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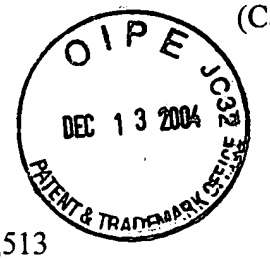
GP1743
IFW Image

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 97,008-W)



In re Application of:		
COPELAND, et. al		Group Art Unit: 1743
Serial No.:	09/931,513	Examiner: Alexander
Filed:	August 16, 2001	
For:	Automated Biological Reaction Apparatus	

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

TRANSMITTAL LETTER

In regard to the above identified application:

1. We are transmitting herewith the attached:
   a. Petition for Four Month Extension of Time
   b. Appeal Brief
   c. Return Receipt Postcard

2. With respect to additional fees:
   a. Attached is a check in the amount of $2,090.00
   ($1590 for four month extension of time and $500 for Appeal Brief)

3. Please charge any additional fees or credit overpayment to Deposit Account No.13-2490. A duplicate copy of this sheet is enclosed.

4. CERTIFICATE OF MAILING UNDER 37 CFR § 1.8: The undersigned hereby certifies that this Transmittal Letter and the paper, as described in paragraph 1 hereinabove, are being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Appeal Brief-Patents Commissioner for Patents, P.O. Box 1450, Arlington, Virginia 22313-1450 on this 9th day of December, 2004.

By : C BC Hughes
A. Blair Hughes
Reg. No. 32,901

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 97,008-W)

In re Application of	)	
	)	
Copeland, et al.	)	
	)	Group Art Unit: 1743
Serial No.: 09/931,513	)	
	)	
Filed: August 16, 2001	)	Examiner: Lyle Alexander
	)	
For: Automated Biological Reaction Apparatus	)	

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This Appeal Brief is submitted in accordance with the requirements of 37 CFR 41.37. The fee required by 37 CFR 41.20(b)(2) is submitted herewith.

I. REAL PARTY IN INTEREST

The real party in interest of this pending application is Ventana Medical Systems, Inc. which is the owner by Assignment of the above-identified U.S. patent application.

II. RELATED APPEALS AND INTERFERENCES

There are no Appeals or Interferences related to the above-identified U.S. Patent Application.

III. STATUS OF THE CLAIMS

This application contains 99 claims.

- Claims 72, 77, 80-85, 87, 89-91, and 98-99 are pending in the application, stand finally rejected, and are the subject of this appeal.
- Claims 1-71 were cancelled from the application without prejudice in a Preliminary Amendment filed with the application on August 16, 2001.
- Claims 74-75, 78-79, 88, 92-93 and 95-97 were cancelled without prejudice from the application in the applicant's Reply to the examiner's March 15, 2002 Official Action.
- Claim 73 was cancelled from the application without prejudice in the applicant's Reply to the examiner's March 19, 2003 Official Action.
- Claims 76, 86 and 94 were withdrawn from consideration in response to the examiner's January 10, 2003 Restriction Requirement.

A copy of currently pending claims 72, 77, 80-85, 877, 89-91, and 98-99, involved in this appeal, are attached hereto as Appendix A.

IV. STATUS OF AMENDMENTS

The examiner finally rejected all pending application claims 72, 77, 80-85, 87, 89-91, and 98-99 in a Final Rejection dated August December 11, 2003. There are no amendments outstanding.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The invention is directed broadly to methods for automatically staining a biological sample. The process of staining a biological sample involves applying different aqueous solutions, alone or in combination, to the biological sample located on a sample support. Useful biological sample supports include but are not limited to a glass microscope slides (claim 90) and slides (claim 99).

The claimed methods begin with a biological sample that is substantially covered by a first aqueous solution. (Specification page 7, line 23 to page 8, line 14). The biological sample and first and subsequently applied aqueous solutions are often heated during processing. Therefore, an

evaporation-inhibiting liquid is applied to the biological sample to cover the sample and the first aqueous solution to prevent evaporation of the first and subsequently applied aqueous solutions and ultimately avoid dehydration of the biological sample. (Specification at page 27, lines 16-19). Next, in step (a) of claim 72 (claim 72 is the only pending independent claim), a reagent is dispensed on the evaporation–inhibiting liquid phase. (See *e.g.*, claims 72, step a). When a reagent is dispensed onto the evaporation–inhibiting liquid phase, the reagent passes through the evaporation–inhibiting liquid phase and into contact the first aqueous solution and biological sample. (Specification page 27, lines 9-11.) A problem with dispensing a reagent onto the evaporation–inhibiting liquid phase and thereafter into contact with the first aqueous solution is that the dispensed reagent and first aqueous solution need assistance in interacting with the biological sample. (Specification page 27, lines 16-18). This problem is solved in the present invention by directing at least one air jet at the surface of the evaporation-inhibiting liquid phase to cause the evaporation–inhibiting liquid phase to move. (See claim 72 – step b). The movement of the evaporation–inhibiting liquid phase is imparted to the underlying first aqueous solution and reagent mixture. Importantly, the claims require moving the evaporation-inhibiting liquid phase "while preserving the biological sample from dehydration or other damage from the air jets." (Specification page 27, lines 16-18.) To prevent sample dehydration, a gentle air jet is directed at the surface of the evaporation–inhibiting liquid phase. This gentle application of an air jet does not disrupt the evaporation-inhibiting liquid phase thereby preserving the underlying solutions and sample. *Id.*

The claimed method is described generally in the specification with reference to at least Figures 17 and 18A-18C. Figures 18A-18C are reproduced immediately below.

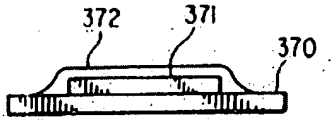


FIG.18A

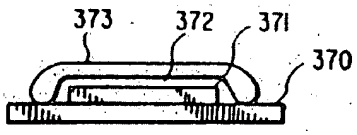


FIG.18B

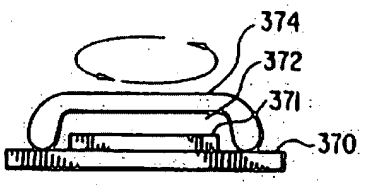


FIG.18C

The specification discusses the claimed method with reference to Figures 18A-18C as follows:

FIG. 18 is a schematic representational cross-sectional view of a slide 370

following the rinse liquid, evaporation inhibitor and reagent application steps. Following the rinse stages (Stage A), the tissue section 371 mounted on slide 370 is covered with a thin residual aqueous layer 372. Following application of the evaporation inhibitor liquid (Stage B), the aqueous layer 372 and tissue section 371 is entirely covered by a layer 373 of the evaporation inhibitor liquid. Aqueous reagent 374, applied to the slide, flows under the evaporation inhibitor layer 373 to cover the tissue section. In the vortex mixing section (Stage C), air jets directed against the surface of the evaporation inhibitor liquid 373 move it and the reagent solution 374 thereunder in a swirling or stirring action on the surface of the fragile tissue section. This gentle stirring achieves increased interaction of reagent with the tissue section while preserving the tissue from dehydration or other damage from the air jets.

(Specification page 27, lines 1-19)

The evaporation-inhibiting liquid phase may be moved with an air stream by a number of different non-limiting methods. For example, the evaporation-inhibiting liquid phase may be moved by directing an air jet to an area on the surface of the evaporation-inhibiting liquid phase between the center of the evaporating-inhibiting liquid phase and the edge of the support medium. (See claim 80). The evaporation-inhibiting liquid phase may be moved with a single gas jet, or with two or more gas jets. If two gas jets are used, then the gas jets may be applied in a variety of directions including opposite one another (claim 82) or at locations between the center of the evaporation-inhibiting liquid phase and the biological support medium.

The specification discloses, with reference to Figure 17, at least the following methods for using an air jet to gently create a vortex in the evaporation-inhibiting liquid layer:

Fig. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action. Pressurized air is supplied to the nozzle channels 350 and 354 by channel 358. The reagent solution covered by a layer 360 of evaporation inhibiting liquid 360 is stirred on the surface of the biological sample by applying at least one gas stream 356 or 357 to an area of the surface of the evaporation inhibiting liquid layer 360 between the center of the evaporation inhibiting layer 360 and the edge of the planar support surface 361 or 362 of the slide 228. The gas stream impacts the surface of the evaporation liquid surface layer 360 and moves the underlying reagent solution in a circular path on the tissue section. Preferably, the reagent solution is stirred on the surface of the biological sample by a vortex formed by applying two gas streams 356 and 347. Stream 356 is directed against an area 363 of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge 361. Stream 357, in a direction opposite to the direction of stream 356, is directed against an area 364 of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge 362.

> Although this method is shown with respect to an evaporation liquid inhibitor covered reagent layer, it will be readily evident that it can be applied to gently stir any liquid layer overlying a fragile substance.

(Specification page 26, lines 7-34).

The evaporation–inhibiting liquid phase is "substantially water-insoluble, substantially water-immiscible and substantially thin or non-viscous. It has a specific gravity less than water, and a boiling point above the process temperature, preferably above 100° C. It should be devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample, that is, the reactions taking place between the reagents and tissue sample on the slide. Preferred evaporation inhibiting liquids are hydrocarbons, optimally non-aromatic saturated hydrocarbons, having from 9 to 18 carbons, most optimally having about 10 to 14 carbon atoms." (Specification page 22, line 30 to page 23, line 10).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The examiner rejected claims 72, 77, 80-85, 87, 89-91 and 98-99 for allegedly being unpatentable under 35 U.S.C. § 103(a) for obviousness over Miller et al. (USP No. 5,225,325) in view of Mazza et al. (USP No. 4,815,978). The examiner also cited Swope (USP No. 5,350,697) in passing in the December 11, 2003 Final Rejection. Therefore, for purposes of this appeal, applicant assumes that the Swope reference is integral to the examiner's final rejection.

The examiner's position is that Miller et al. discloses all of the features of the claimed invention except for using at least one stream of air applied to the surface to facilitate solution mixing. (See December 11, 2003 Final Rejection at page 2). The examiner relies upon Mazza et al. to supply the missing teaching. The examiner cites Mazza et al. for teaching that it is desirable to mix the sample with a jet of air to prevent splashing of the sample that would waste the sample and would contaminate the lab. Finally, the examiner relies on Swope et al. for teaching that cuvettes and slides are interchangeable for optical analysis in immunoassay art. (See page 4 of the 3/19/2003 Official Action.) The examiner concludes that it would have been within the scope of the art to modify Miller et al. in view of Mazza et al. to use an air jet to mix samples according to the claimed methods.

VII. ARGUMENT

The Examiner's Obviousness Rejection Cannot Be Sustained Because Two Of The Three References Cited In The Examiner's Obviousness Rejection - The Miller et al. and Swope et al. References - Are Not Available As Prior Art To The Presently Claimed Invention

Two of the three references cited by the Examiner – Miller et al. and Swope et al. - are not available as prior art to the claimed invention under 35 USC §102. Therefore, the Examiner's obviousness rejection, which relies on each reference, cannot be sustained by the Board and all pending claims must be allowed.

A. March 2, 1990 Is The Priority Date Of The Presently Claimed Invention

The present application claims priority to U.S. Application Serial No. 07/488,601, filed on March 2, 1990. (*See* Application Filing Receipt – Tab A – Evidence Appendix). Moreover, the invention of pending claims 72, 77, 80-85, 87, 89-91 and 98-99 is fully supported by the specification of Application Serial No. 07/488,601. A copy of 07/488,601 is attached to this Brief at Tab B of the Evidence at Appendix. The presently claimed invention is fully disclosed in the '601 application a summarized below:

- Page 6, lines 24-26.
- Page 7, line 27 to Page 8, line 4.
- Figure 16 and the accompanying description at page 23, lines 15-25.
- Figure 17 and the accompanying description at page 23, line 26 to page 24, line 19.
- Figure 18 and the accompanying description at page 24, line 20 to page 25, line 4.
- Page 38, line 31 to page 39, line2.
- Claims 45-47.

B. Miller et al. Is Not Available As Prior Art To The Claimed Invention

March 2, 1990 is the earliest priority date claimed in Miller et al. U.S. Patent No. 5,225,325. The Miller et al. priority date is identical to the earliest claimed priority date of the present application. Therefore, Miller et al. is not prior art to the present application under any paragraph of 35 U.S.C. § 102. As a result, the examiner's obviousness rejection of claims 72, 77, 80-85, 87, 89-91 and 98-99, which is premised upon the combination of the Miller et al., Mazza et al., and Swope et al. patents cannot be sustained by the Board because Miller et al. is not prior art to the

claimed invention.

C. Swope et al. Is Not Available As Prior Art To The Claimed Invention

August 28, 1990 is the earliest priority date claimed in Swope et al. U.S. Patent No. 5,350,697. This date is after the March 2, 1990 earliest claimed priority date of the present application. Therefore, Swope et al. is not available under any paragraph of 35 U.S.C § 102 as prior art to the presently claimed invention. As a result, the examiner's obviousness rejection of claims 72, 77, 80-85, 87, 89-91 and 98-99, which is premised upon the combination of the Miller et al., Mazza et al., and Swope et al. patents cannot be sustained by the Board because Swope et al. is not prior art to the claimed invention.

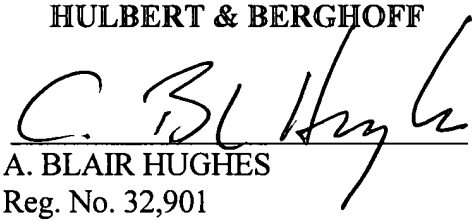
CONCLUSION

The examiner's obviousness rejection of claims 72, 77, 80-85, 87, 89-91 and 98-99 cannot be sustained by the Board because two of the three references relied upon by the examiner in rendering the final obviousness rejection are not available as prior art against the claimed invention. Without the two references, the examiner has failed to establish a *prima facie* case of obviousness and all pending application claims must be allowed.

Respectfully submitted,

McDONNELL BOEHNEN
HULBERT & BERGHOFF

Dated: December 9, 2004

By: 

A. BLAIR HUGHES
Reg. No. 32,901
312-913-2123

APPENDIX A

Listing of Claims:

72. In a method for automatically staining a biological sample, the biological sample being on a support medium and substantially covered by a first aqueous solution, and an evaporation-inhibiting liquid phase covering the first aqueous solution, the improvement comprising:

a) dispensing a reagent onto the evaporation-inhibiting liquid phase; and

b) sending at least one stream of air to a surface of the evaporation-inhibiting liquid phase to move the evaporation-inhibiting liquid phase, thereby stirring the reagent with the biological sample on the support medium while preserving the biological sample from dehydration from the stream of air.

77. The method of claim 72, wherein said biological sample comprises tissue.

80. The method of claim 72, wherein the stream of air is directed to an area on the surface of the evaporation-inhibiting liquid phase between a center of the evaporation-inhibiting liquid phase and an edge of the support medium.

81. The method of claim 80, wherein the at least one gas stream moves the reagent in a circular path.

82. The method of claim 72, wherein two streams are applied; and

wherein the two streams are applied in opposite directions.

83. The method of claim 72, wherein two streams are applied;

wherein the first stream is directed against a first area of the surface of the evaporation-inhibiting liquid phase between a center of the evaporation-inhibiting liquid phase and a first edge of the support medium; and

wherein the second stream is directed against a second area of the evaporation-inhibiting

liquid phase between the center of the second solution and a second edge of the support medium.

84. The method of claim 72, wherein stirring said evaporation-inhibiting liquid phase comprises directing at least one stream of air at an angle to the surface of said evaporation-inhibiting liquid phase and maintaining it long enough to cause a rotation of the evaporation-inhibiting liquid phase.

85. The method of claim 84, wherein stirring said evaporation-inhibiting liquid phase comprises creating a vortex in the evaporation-inhibiting liquid phase.

87. The method of claim 72, wherein the step of sending the stream of air causes kinetic motion to be transferred into said first aqueous solution.

89. The method of claim 87, wherein said biological sample comprises polynucleic acid molecules.

90. The method of claim 87, wherein said support medium is a glass microscope slide.

91. The method of claim 87, wherein said evaporation-inhibiting liquid phase is a hydrocarbon having from about 9 to about 18 carbon atoms.

98. The method of claim 72, wherein the step of stirring the evaporation-inhibiting liquid phase accelerates rate of dispersal of reagent to the biological sample covered by the aqueous solution.

99. The method of claim 72, wherein the support medium is a slide.

Evidence Appendix

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/931,513	08/16/2001	1743	1528	97,008-W	37	97	15

CONFIRMATION NO. 5062

20306
MCDONNELL BOEHNEN HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
SUITE 3200
CHICAGO, IL 60606

FILING RECEIPT

OC000000006577829

Date Mailed: 09/19/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

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Charles D. Lemme, Tucson, AZ;
Phillip C. Miller, Tucson, AZ;
William L. Richards, Tucson, AZ;
Wayne A. Showalter, Tucson, AZ;

Domestic Priority data as claimed by applicant

THIS APPLICATION IS A CON OF 09/452,309 12/01/1999
WHICH IS A CON OF 08/906,678 08/05/1997 ABN
WHICH IS A CON OF 08/479,415 06/06/1995 PAT 5,654,200
WHICH IS A DIV OF 08/352,966 12/09/1994 PAT 5,595,707
WHICH IS A CON OF 07/924,052 08/31/1992 ABN
WHICH IS A CIP OF 07/488,601 03/02/1990 ABN

Foreign Applications

If Required, Foreign Filing License Granted 09/19/2001

Projected Publication Date: 12/27/2001

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Automated biological reaction apparatus

Preliminary Class

436

Data entry by : SEDIQEE, AHMADULLAH **Team :** OIPE **Date:** 09/19/2001

FIELD OF THE INVENTION

This invention relates an improved biological reaction platform which can be used for a wide variety of assays, for example, automatic immunostaining of tissue sections, *in situ* DNA analysis, immunoassays such as ELISA, and the like. The automatic device of this invention can be used to process a large number of samples such as tissue sections mounted on slide surfaces using agents and protocols preselected by the operator, while maintaining the slide surfaces in a substantially horizontal plane throughout the incubation cycles.

BACKGROUND AND OBJECTS OF THE INVENTION

Immunostaining and *in situ* DNA analysis are useful tools in histological diagnosis and the study of tissue morphology. Immunostaining relies on the specific binding affinity of antibodies with epitopes in tissue samples, and the increasing availability of antibodies which bind specifically with unique epitopes present only in certain types of diseased cellular tissue. Immunostaining requires a series of treatment steps conducted on a tissue section mounted on a glass slide to highlight by selective staining certain morphological indicators of disease states. Typical steps include pretreatment of the tissue section to reduce non-specific binding, antibody treatment and incubation, enzyme labeled secondary antibody treatment and incubation, substrate reaction with the enzyme to produce a fluorophore or chromophore highlighting areas of the tissue section having epitopes binding with the antibody, counterstaining, and the like. Each of these steps is separated by multiple rinse steps to remove unreacted residual reagent from the prior step. Incubations are conducted at elevated temperatures, usually around 40°C,

and the tissue must be continuously protected from dehydration. *In situ* DNA analysis relies upon the specific binding affinity of probes with unique nucleotide sequences in cell or tissue samples and similarly involves a series of process steps, with a variety of reagents and process temperature requirements.

Automated systems have been explored to introduce cost savings, uniformity of slide preparation, and reduction of procedural human errors. Stross, W. et al, *J.Clin.Pathol.* **42**:106-112 (1989) describes a system comprising a series of baths positioned under the circumference of a circular, rotatable disc from which slide trays are suspended. The disc is lifted to lift slide trays from their baths, turned to position the slide trays above the next consecutive bath, and lowered to immerse the slide trays in the baths. This operation can be automated with suitable timers and switches. This system exposes each of the slides to the same treatment and relies on dipping for application of reactants and rinsing.

Stark, E. et al, *J.Immunol.Methods.* **107**:89-92 (1988) describes a microprocessor controlled system including a revolving table or carousel supporting radially positioned slides. A stepper motor rotates the table, placing each slide under one of the stationary syringes positioned above the slides. A predetermined volume of liquid, determined by a dial, is delivered to a slide from each syringe. Microprocessor controls are provided.

Cosgrove, R. et al, *ACL.* pp 23-27 (December, 1989) describe an immunostaining apparatus for auto-pipetting reagents into a slide well from a carousel holding up to 18 reagent vials. Below each well, a coverplate spaced from the surface of each slide provides cover and defines a reagent flow channel. The slides are suspended at a

steep angle. Reagent from the well flows downward over the slide surface. A row of slides are suspended for sequential treatment. Washing is accomplished by a 3 to 4 minute continuous running wash over the sample, yielding an estimated 20:1 wash/reagent ratio.

Brigati, D. et al, *J.Histotechnology* **11**:165-183 (1988) and Unger, E., Brigati, D. et al, et al, *J.Histotechnology.* **11**:253-258 (1988) describe the Fisher automated work station using capillary gap technology. A coverplate is placed over the slide, forming a capillary gap. Liquid is introduced into the capillary gap by placing the lower edge of the plate-slide pair in a liquid. Liquid is removed by placing the lower edge of the plate-slide pair on a blotter. The system is further described in U.S. Patents 4,777,020, 4,798,706 and 4,801,431. The previously known devices are limited in their performance and unable to satisfy the needs for automated, high precision immunohistology.

It is an object of this invention to provide a device which provides more rapid, reliable and more reproducible results than standard methods; can perform any standard immunochemical assay including assays relying on immunofluorescence, indirect immunoassay procedures, peroxidase anti-peroxidase methods, or avidin-biotin technology; preforms all steps of the immunohistochemical assay irrespective of complexity or their order, at the time and temperature, and in the environment needed; and is cost effective in terms of equipment, reagent and labor costs.

SUMMARY OF THE INVENTION

The automated biological processing apparatus of this invention comprises a reagent carousel cooperating with a sample support carousel to apply a sequence of

preselected reagents to each of the samples with interposed mixing, incubating, and rinsing steps cooperating therewith. The carousel slide support has a plurality of slide supports thereon and drive means engaging the carousel slide support for consecutively positioning each of a plurality of slide supports in a reagent receiving zone. The reagent carousel has a plurality of reagent container supports thereon and drive means engaging the carousel for rotating the carousel and positioning a preselected reagent container support in a reagent supply zone. The apparatus has a reagent delivery actuator means positioned for engaging a reagent container positioned on a container support in the reagent supply zone and initiating reagent delivery from the reagent container to a slide supported on a slide support in the reagent receiving zone.

The apparatus preferably has bar code readers positioned to read bar codes on the sample containers or slides and on the reagent containers. The carousels have homing systems containing a detectable component and a proximity detector therefor for indexing the position of the reagent containers and slides.

The apparatus preferably has a heating chamber means surrounding the carousel slide support for heating slides supported thereon to a predetermined temperature. The heating chamber means includes a hot gas manifold having a plurality of hot gas outlets positioned above the slide supports. The heating chamber means includes a temperature sensor and a hot gas control means connected to the temperature sensor for increasing heat supplied to gas flowing through the manifold and for increasing the hot gas flow rate if further heat is required to maintain the heating chamber at a preselected temperature. The temperature sensor is a thermistor, the tip thereof being

enclosed in a heat sensitivity reducing jacket. The hot gas control system includes two heating components with separate controls and a speed control for the hot gas fan.

The drive means engaging the carousel slide support is also a means for consecutively positioning each of a plurality of slide supports in a plurality of adjacent rinse zones, an evaporation control liquid and reagent receiving zone, a vortex mixing zone, and an incubation zone. Rinse spray means are positioned adjacent each rinse zone for applying rinse liquid to the surface of slides positioned in the respective rinse zone. The apparatus slide receptors are pivotally mounted thereon for pivotal motion from a horizontal slide incubation position to a tilted slide draining position following each pulse of rinse liquid.

The apparatus preferably has a volumetric pump means, and a reagent delivery actuator means positioned for activating the volumetric pump means, thereby effecting delivery of reagent from a reagent container by the volumetric pump to the reagent delivery zone. An evaporation inhibitor liquid application means is positioned adjacent the reagent delivery zone.

Vortex agitation means are positioned adjacent the agitation zone for stirring reactants on a slide supported in the vortex agitation zone.

The carousel slide support comprises a support plate having distal and proximal ends, and a slide support surface. The distal end has raised terminal and lateral distal guide tabs with guide termini. The proximal end has first and second lateral guide tabs with opposed slide engaging surfaces for engaging the lateral edges of a slide. The guide termini are lower than the upper slide surface plane.

An improved biochemical method of this invention with increased sample dehydration protection comprises carrying out a biochemical reaction under a layer of evaporation inhibiting liquid. The improvement comprises (a) covering the sample with an aqueous surface layer by applying an aqueous solution to a planar support surface adjacent a biological sample mounted thereon; and (b) covering the aqueous surface layer with an evaporation inhibiting liquid layer by applying the evaporation inhibiting liquid to the planar support surface adjacent the biological sample in an amount sufficient to form a continuous layer of evaporation inhibiting liquid over the sample. The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially non-viscous; has a specific gravity less than water, and a boiling point above 50°C; and is devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample. The biological sample can then be optionally treated (c) with an aqueous reagent solution by applying the reagent solution to the planar support surface adjacent the biological sample. The reagent solution flows to the biological sample under the evaporation inhibiting liquid layer, and the sample is continuously protected from dehydration by the evaporation inhibiting layer.

In another aspect of this invention, the reagent solution is stirred on the surface of the biological sample by applying at least one gas stream to an area of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the edge of the planar support surface, the gas stream having a central axis forming an acute angle with the planar support surface. Preferably, the reagent

solution is stirred by a vortex formed by applying two off-center gas streams, flowing in opposite directions, to the surface of the evaporation inhibiting liquid layer.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a left front, isometric view of the automated immunostaining apparatus of this invention, with the cabinet shell removed.

Fig. 2 is an exploded right front isometric view of the apparatus shown in Fig. 1.

Fig. 3 is a partial exploded left front isometric view of the apparatus shown in Fig. 1.

Fig. 4 is a partial exploded right rear isometric view of the apparatus shown in Fig. 1.

Fig. 5 is a top view of a pivotally mounted slide support.

Fig. 6 is an isometric view of the underside of the slide support component.

Fig. 7 is a side view of the pivotally mounted slide support of Fig. 5 showing the tipper and mounting details.

Fig. 8 is an isometric view of the mounted slide support of Fig. 7 in the untipped position.

Fig. 9 is an isometric view of the mounted slide support of Fig. 7 in the tipped position.

Fig. 10 is a distal end view of the mounted slide support in the tipped position.

Fig. 11 a fragmentary top view of the slide support carousel showing details of the slide treatment stations.

Fig. 12 is a schematic cross-sectional view of a rinse station taken along the line A-A in Fig. 11, showing details of rinse liquid flow on a slide.

Fig. 13 is a top schematic view of the rinse stations showing details of the rinse liquid distribution on slides being treated therein.

Fig. 14 is an isometric view of the slide treatment bar code reading, rinse, reagent receiving and vortex mixing stations.

Fig. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent receiving station, taken along the line B-B in Fig. 11.

Fig. 16 is a cross-sectional view of the vortex mixing assembly, taken along the line C-C in Fig. 11.

Fig. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action.

Fig. 18 is a schematic representational cross-sectional view of a slide following the rinse liquid, evaporation inhibitor and reagent application steps.

Fig. 19 is a cross-sectional view of a rinse liquid container and associated heating components.

Fig. 20 is a bottom, isometric view of a reagent container support tray.

Fig. 21 is a fragmentary cross-sectional view taken along the line D-D in Fig. 11 showing the slide carousel metal proximity sensor indexing system of this invention.

Fig. 22 is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention.

Fig. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention.

Fig. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention.

Fig. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention.

Fig. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention.

Fig. 27 is schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention.

DETAILED DESCRIPTION OF THE INVENTION

The automated immunostaining apparatus of this invention preforms all steps of immunohistochemical and *in situ* DNA assays irrespective of complexity or their order, at the time and temperature, and in the environment needed. Specially prepared slides containing a bar code identifier and a mounted tissue section are placed in special support on a carousel, subjected to a preprogrammed sequence of reactions, and are removed from the carousel, ready for coverslipping and histological examination. For purposes of clarity of the following description of the apparatus of this invention and not by way of limitation, the apparatus will be described in terms of immunohistochemical processes.

Fig. 1 is a front right, isometric view of the automated immunostaining apparatus of this invention, with the cabinet shell removed. Liquid and air supply tubing and electrical wiring connecting the respective components are conventional, well known in the art, and are omitted from the drawings for purposes of clarity. The apparatus has an upper section 2, intermediate section 4 and lower section 6. In the upper section 2, reagent bottle support carousel 10 is mounted for rotation about its central axis 7 on upper support plate 8. Reagent bottles 12 required for the immunohistochemical reactions to be conducted during slide treatment cycle are supported by the carousel 10, mounted

in reagent bottle receptors 11. These receptors are configured to receive volumetric pump outlet tube 307, shown in detail in Fig. 14. The receptors 11 are preferably equally spaced in a circular pattern axially concentric with the carousel axis 7. The number of receptors provided should be sufficient to accommodate the number of different reagent bottles required for a cycle or series of cycles. Twenty-five receptors are shown, but the number can be smaller or greater, and the diameter of the carousel 10 can be increased to accept a larger number of reagent containers. The carousel is rotated by the stepper motor 14 drive belt 16 to a position placing a selected reagent bottle 12 in the reagent deliver position under the air cylinder reagent delivery actuator 18 over a slide to be treated with reagent. Reagent tray motor driver 20 is connected to stepper motor 14.

The intermediate section 4 comprises support plate 22 upon which the slide support carousel 24 is rotatably mounted. The carousel 24 supports slide supports 26. Heated air supply chamber 28 communicates with the heated air supply manifold 30 supported on the underside of plate 8 and lid heated air supply manifold 31 mounted on the upper plate 8 by hinged supports 33. The support plate also supports the conventional computer board 32, LCD display 34, disk drive 35 and computer 36 used to operate the apparatus. Air pressure regulator 38 regulates the pressure of air delivered to the evaporation inhibitor and rinse liquid delivery systems described in Fig. 22.

The lower section 6 includes support plate 40 upon which are supported accessories such as power supply filter 42 and hot water supply 44.

Fig. 2, Fig. 3 and Fig. 4 are exploded right front, left front and right rear isometric views of the apparatus shown in Fig. 1. Tipper air cylinders 46 are positioned on support plate 8. These cylinders are aligned to actuate a tipper cam surface 148 against a slide support tab surface 112 shown in detail in Figs. 8, 9 and 10.

In the intermediate section 4, the stepper motor 48 rotates the slide support carousel 24, engaging drive belt 25 (Figs. 3 and 4) engaging the perimeter of the carousel. Splash guard 50 is a wall which surrounds the sides, back and part of the front of the carousel, defines the heating zone and contains the liquid spray and droplets produced in the processing. It extends upward from the intermediate plate 22 to a position adjacent the upper plate 8, leaving an air flow gap between the upper edge of the splash guard and the underside of the plate 8. Mounted on the underside of upper support plate 8 above the carousel 24 and within the perimeter of the splash guard 50 is the heated gas supply manifold 30. Heated air is directed downward and over the slide supports 26 by holes 336 (Fig. 15) in the manifold 30. The heated air then passes upward over the top of the splash guard and exits the device. Extending upward through central opening 52 of carousel 24 into the heated air supply chamber 28 is the fan shroud 54 and axially positioned fan 56. The fan is positioned over air vents 57 in the bottom plate 22. The annular waste liquid sump 58 surrounds the shroud 54, below liquid outlet ports 292 (Fig. 14), and is supported on the bottom of plate 22. The waste reagent and rinse liquids are collected in the sump and passed to a drain through an outlet tube in the sump bottom (not shown).

Rinse and liquid coverslip spray blocks 60 are supplied with liquid through conventional solenoid valves 62.

Temperature controller 66, mounted on support plate 22, controls the heat energy supplied to the heated water container 44. Temperature controllers 68 and 70, mounted on support plate 40 (Fig. 4), control the temperature of the air in the air heater supply chamber 24 by controlling energy supplied to respective annular heater elements 331 and 332 (Fig. 15). Slide carousel stepper motor driver 72 and relay 74 operate stepper motor 48. Power supplies 76 and 78 provide power to the stepper motors and control systems. Air compressor 80 supplies air to the air filter 82 and air pressure regulators 38, 64 and 86.

Fig. 5 is a top view of a mounted slide support 26 with slide edges 100 and 101 represented by dashed lines. The slide support 26 has a support plate 102 with a distal end 103 and a proximal end 104. The distal end 103 has a raised terminal guide end tab 106 and two lateral guide tabs 108 and 110 with the upper edges constituting guide tab termini. The distance between the upper surface of the slide support and the guide tab termini (the elevation above the upper surface) is less then the thickness of a conventional microscope slide. The proximal end 104 of the slide support has opposed lateral guides 112 and 114 for engaging the lateral edges of a slide and a terminal end tab 115 for engaging the proximal end of a slide. The proximal end 104 of the slide support has an inflexible support portion 116 and a flexible arm 118 with opposed lateral edges 120 and 122. The distance between the slide edge engaging surfaces 111 and 113 of the guide tabs 112 and 114 is less than the width of a slide to be supported on the support. A

standard slide has a width of 1 inch or 25 mm, and the preferred distance between the slide edge engaging surfaces of the tabs for supporting a standard slide is from 20 to 24 mm. The flexure of arm 118 permits positioning of the slide between the lateral guide tabs and terminal end tabs. The distance between the opposing tab surfaces 111 and 113 causes the slide support to apply a positive pressure on the edges of a slide, retaining the slide securely on the support during the tilting and other processing steps. The upper surface of the support plate 102 is preferably planar and smooth so the wet slide rests closely on the surface, and surface tension will resist vertical movement of the slide from the support surface.

Fig. 6 is an isometric view of the underside of the slide support component. The inflexible portion 116 has an integral pivot support 124 which reinforces the inflexible portion to prevent flexure. The flexible arm 118 has sufficient depth or thickness to limit the flexural movement of the arm to a horizontal direction. This insures effective cooperation and pressure between the guide tab 112 on the inflexible portion and the guide tab 114 on the flexible portion to assist in retaining the slide in place on the support during the tipping operation described in detail hereinafter.

Fig. 7 is a side view of a mounted slide support showing the tipper and mounting details. The upper pivot support 124 is pivotally mounted on the lower pivot support 126. Pivot support 126 has upward extending projections 128 and 130 which engage the ends 132 and 134 of the pivot support 124. Pivot pin 136 extends through an axially aligned hole in projection 128 into an axially aligned receptor hole 138 in the opposing end 132 of the pivot support 124. At the opposite end, axially

concentric with pivot pin 136, pivot pin 140 extends through a hole in projection 128 (not shown) into a respective receptor hole in the opposing end 134 of the pivot support 124. The slide support 102 is thus mounted for pivotal motion around the common pivot axis of the pins 136 and 140. Bias spring 142 is supported on pin 134, one end 141 pressing against the lower abutment surface 143 of inflexible support portion 151, and the other end 144 bearing against spring stop groove 145 in the spring stop 146. The tip 148 of tipper 150 is positioned above the upper surface of guide tab 112 when the slides are positioned in a rinse station, described in greater detail hereinafter with respect to Fig. 13.

The pivot pins 136 and 140 support the upper surface of the slide support 102 at a small angle 'a' from the horizontal plane to aid liquid flow toward the distal end during treatment. Angle 'a' is preferably in the range of from 0.3 to 1.0°. The upper slide support surface 151 and the upper slide surface 152 (dotted line) supported thereon are thus maintained at a slight incline from the horizontal plane downward toward the distal end of the slide.

Fig. 8 is an isometric view of a mounted slide support in the untipped position, Fig. 9 is an isometric view of the mounted slide support in the tipped position, and Fig. 10 is a distal end view of the mounted slide support in the tipped position. Vertically downward pressure of the tipper tip 148 against the upper guide tab surface 154 rotates the slide support 102 about the pivot axis 156 defined by the pivot pins 136 and 140. The tipper axis preferably lies in a vertical plane through the midpoint of distal end 103 and the left edge proximal end 104 of the slide support. The tipping action tilts the slide surface to an angle 'c' of

approximately 60° from the vertical (Fig. 10). It sharply lowers the left distal corner 158 and sharply raises the right proximal corner 160, breaking the liquid meniscus on the slide surface and directing the liquid flow 159 to the corner 158 and off the surface of the slide into drain hole 292. The pivotal movement increases the pressure of the spring against spring stop groove 145, and as the tipper 150 is raised, the slide support returns to its original position. The slide support return pivot motion is terminated when the lower right distal corner 162 of the slide support 102 abuts stop surface 164 of the pivot support 126.

Fig. 11 a fragmentary top view of the slide support carousel showing details of the slide treatment stations. Rinse nozzle blocks 200, 202 and 204 and the adjacent respective slides 206, 208 and 210 define successive rinse zones, details of which are shown in Figs. 12-14. Evaporation inhibitor liquid application block 212 and the adjacent slide 214 define the evaporation inhibitor and reagent application zone, details of which are shown in Fig. 15. Air cylinder reagent delivery actuator 18, supported by support arm 216, contacts reagent bottle 218, directly over slide 214. Vortex mixer air jet blocks 220, 222 and 224 are positioned adjacent slides 226 and 228 in the agitation zone, details of which are shown in Fig. 16 and 17. The hanger 352 is mounted on the tip of blocks 220 and 222 and supports suspended block 224. Pressurized air is delivered to block 224 by conduit 358. As the slide support carousel 24 positions each slide for successive treatment in the rinse zones, evaporation inhibitor and reagent application zone, and agitation zones (counter-clockwise movement of the carousel), the tissue sections on each slide are first rinsed and then covered with evaporation inhibitor.

Reagent is applied from a preselected reagent bottle to the tissue through the evaporation inhibitor layer, and the reagent is agitated through the evaporator inhibitor layer by the vortex mixer. Each slide then is moved around the incubation zone, a circular path traveled by the carousel, heated with hot air from the heated air manifold, and the reagent reacts with the sample. As the carousel continues to increment around the circle, each slide is returned to the rinse stations, etc, for application of the next reagent required in the reaction. This entirely automated process continues until the desired reactions are completed.

Bar code reader 231 above slide 205 reads a slide bar code 233 (Figs. 13 and 17) on each slide. The slide bar codes identifies the slide sample and the particular immunohistochemical process required for that sample. This information is fed into the computer and correlated with the indexed position of that slide with respect to "home", to control the sequence of reagent chemicals to be applied to that slide in the reagent application zone.

Fig. 12 is a schematic cross-sectional view of a rinse station taken along the line A-A in Fig. 10, showing details of rinse liquid flow on a slide. Rinse block 200 mounted on plate 22 has a heated rinse liquid supply channel 230 communicating with rinse liquid nozzle 232. The slide 234 has a sloping surface at an angle '*a*', being supported on the sloping surface of the slide support 124. The slide 234 has a rinse liquid impact zone 236 adjacent the proximal end 100 between the bar code 233 and the sample 238. The impact zone 236 is at a higher elevation than the tissue section 238 supported adjacent the distal end 103. The nozzle axis 240 has an angle '*b*' which directs liquid against the slide surface impact zone 236. The impact zone 236 is above the tissue

section 238 on the sloped surface of slide 240, and the rinse liquid stream 242 flows across the upper surface of the tissue section 238 toward the distal end 103. The angle '*b*' preferably has an angle of from 15 to 35°, and the distance between the exit of nozzle 232 and the slide is selected to direct the rinse liquid precisely on the impact zone 236, avoiding disturbance of the fragile tissue section 238.

The lower carousel is rotated above the plate 22, the outer periphery being supported by low friction slide bearings 244 arrayed in an axially concentric circular path on plate 22 under the outer periphery of carousel 24.

Fig. 13 is a top schematic view of the rinse stations showing details of the rinse liquid distribution on slides being rinsed therein. Slides 238, 246, and 248 are positioned in the path of heated rinse solutions (dotted lines) from rinse station blocks 200, 202 and 204. Fragile tissue sections 238, 250 and 252 are positioned adjacent the distal end of the slides. The rinse liquid impact zones 236, 254 and 256 are positioned between the tissue sections and the proximal ends of the slides, to avoid direct impact of the liquid jets from the rinse block nozzles. The rinse nozzles on each block are preferably 11.5 mm apart. Rinse block 200 has right offset nozzles 232 and 258 (offset 2 mm to the right of center) supplied by channel 230 connected to supply tubing 260. This directs the rinse fluid toward the right surface of the slide, effecting a transverse flow path across the tissue section 238 to the distal end drain corner 158. Rinse block 202 has symmetrical nozzles 262 and 264 supplied by channel 266 connected to supply tubing 268. The symmetrical nozzle configuration effects a central flow path across the tissue section

250. Rinse block 204 has left offset nozzles 270 and 272 (offset 2 mm to the left of center) supplied by channel 274 connected to supply tubing 276. The left offset nozzles 270 and 272 direct a rinse flow path down the left side of the tissue section 252. The nozzle patterns provide effective rinse solution flow distribution across all portions of the tissue section surface as the slide is treated in each successive rinse section.

Fig. 14 is an isometric view of the rinse stations, a evaporation inhibiting liquid and reagent application station, and agitation stations, showing details of the slide tipping action in the rinse sections. Tipper air cylinders 46 (Fig. 3 and 4) comprises three conventional air cylinders 278, 280 and 282 with internal pressurized air activated pistons or equivalent actuators. Pressurized air delivery to the cylinders causes respective tippers 148, 284 and 286 to move downward, pressing against respective slide support tabs 112, 288 and 290. Three tipper positions are shown to illustrate the action thereof. Tipper 148 is shown in the fully withdrawn or resting position, and slide 206 is in the rinse solution receiving position. After application of heated rinse solution, the tipper descends through an intermediate position shown by tipper 284 and slide support 208, to the drain position shown by tipper 286 and slide support 210. Liquid drains from the left distal corner (lowest corner) into a drain hole 292.

In each rinse station, the sample is treated with a repeated, preferably at least seven, rinse cycles. Each rinse cycle comprises application of approximately 500 μL of heated rinse solution in a short pulse (120 msec) to the slide, followed by tipping the slide to drain away the rinse solution. An estimated 150 μL of liquid remains on the slide after draining. These rinse cycles

are repeated in each rinse station. The short rinse pulse followed by draining prevents the formation of a equilibrium solute boundary layer and greatly increases the rinse efficiency, overcoming the boundary problems present in the prior art rinse methods. Assuming that 150 μL of rinse solution is left after each draining step, a 23 percent dilution is achieved with each rinse cycle. Thus the effective dilution in the combination of the three rinse stations is estimated to be 0.2 parts per trillion, many orders of magnitude more effective than prior art, biochemical rinse procedures. This greatly increases the sensitivity of the immunohistological process.

Fig. 15 is a schematic, fragmentary cross-sectional view of the evaporation inhibiting liquid and reagent application station, taken along the line B-B in Fig. 11. Evaporation inhibitor liquid distributor block 212 has a supply channel 293 and outlet nozzles 294.

The evaporation inhibiting liquid is substantially water-insoluble, substantially water-immiscible and substantially thin or non-viscous. It has a specific gravity less than water, and a boiling point above the process temperature, preferably above 100°C. It should be devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample, that is, the reactions taking place between the reagents and tissue sample on the slide. Preferred evaporation inhibiting liquids are hydrocarbons, optimally non-aromatic saturated hydrocarbons, having from 9 to 18 carbons, most optimally having about 10 to 14 carbon atoms.

A small quantity of evaporation inhibitor liquid is directed by nozzle 294 in a inhibitor liquid stream 296 to an impact zone 298 on the slide between the tissue

sample 299 and the proximal end 100 of the slide, so that the tissue sample is not disturbed. The evaporation inhibitor liquid flows across the surface of the water layer on the wetted tissue, forming a thin evaporation inhibiting film over the aqueous layer which usually covers most of the upper surface of the slide. The tissue is now ready for application of reagent.

The reagent delivery combination includes a conventional air cylinder 18 or equivalent actuator having an internal pressurized air activated piston. It is supplied with pressurized air by tubing 300. Air cylinder 18 is supported by plate 216 and post 302 mounted on upper plate 8. Delivery of pressurized air to the cylinder 18 causes rod 304 and its attached foot 306 to move downward against a reagent container 12 positioned in the reagent delivery zone. Downward movement of reagent container 12 causes emission of a precise volume of reagent liquid 310. Suitable volumetric pumps are available from S. A. Valois and are described in U.S. Patent 4,245,967 and French patent 2,528,122.

The reagent carousel support 314 is the drive plate which supports the reagent carousel and rotates it about its axis to place a predetermined reagent bottle in the reagent delivery zone. An axially concentric circular array of low friction slide bearings 316, mounted on the upper plate 8, are positioned under the outer edge of the reagent support carousel.

The volume of aqueous reagent 310 impacts the evaporation inhibitor surface film between the impact zone 298 and the upper edge of the tissue sample 299, passing through the film to the aqueous layer beneath the film and reaching the slide surface. The reagent then flows across the tissue section under the covering film

of evaporation inhibiting liquid. In this sequence, immediately after leaving the rinse stations, the slide is covered with a protective film to prevent any dehydration of the tissue section. The reagent solution is then applied to the protected tissue. Dehydration of the tissue section would irreversibly alter its physical and chemical characteristics and impair the immunohistochemical reactions. Dehydration is a constant hazard because of the constant flow of heated air over the slides required to maintain them at the desired temperature. The heated air temperature is determined by the requirements of the biochemical processes required by the process. It is slightly above 40°C, preferably about 45°C, for immunochemical reactions and can be as high as from 93 to 97°C for *in situ* DNA hybridization reactions.

Fig. 15 also shows detailed elements of the heated air supply chamber 28 shown in Fig. 1. Air is moved upward into the central intake manifold chamber 330 and through annular heating coils 331 and 332 mounted on annular air passageway plate 333, to heat the air to a temperature slightly above 40°C, preferably about 45°C. A higher temperature can be provided as needed for *in situ* DNA hybridization procedures. The heated air passes through the outlet manifold chamber 334 and out the outlet passageways 336 in the lower plate 338. Annular, axially concentric inner and outer heated air flow control curtains 340 and 342 direct the heated air downward over the slide surface. The reagent 310 falls through manifold passageway 344 to the slide surface.

The air temperature is monitored by heat sensor 345 positioned in the path of the heated air. A preferred heat sensor is a thermistor encased in a heat sensitivity adjusting jacket 347 which reduces the sensitivity of the

thermocouple and approximates the thermal mass of the slides.

Bar code reader 346 can be mounted on post 302, positioned to scan a bar code 348 on the reagent container 12. Bar code 348 identifies the contents of the reagent bottle. At the beginning of a slide treatment operation, the reagent carousel 10 is rotated by the bar code reader 346, and the bar code on each reagent bottle is scanned. The scanned information is fed to the computer and correlated with the indexed position of the reagent carousel. This information is used to rotate the reagent carousel 10 to place the correct reagent bottle in the application zone for each slide treatment step for each slide.

Fig. 16 is a cross-sectional view of the vortex mixing assembly, taken along the line C-C in Fig. 11. Outer vortex jet block 222, mounted on plate 22, has an pressurized air supply channel 350 and nozzle 351. Nozzle hanger 352 is mounted on the top of vortex block 22 and supports suspended inner vortex air jet nozzle block 224. Channel 354 supplies nozzle 355 in block 224 with pressurized air. Nozzles 351 and 355 have central axes which form angles '*d*' and 'e' of from 5 to 15° with the horizontal, directing air jets 356 and 357 toward the slide surface at the corresponding acute angles.

Fig. 17 is a top schematic view of the vortex mixing zone, showing details of the vortex mixing action. Pressurized air is supplied to the nozzle channels 350 and 354 by channel 358. The reagent solution covered by a layer of evaporation inhibiting liquid 360 is stirred on the surface of the biological sample by applying at least one gas stream 356 or 357 to an area of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the edge

of the planar support surface 361 or 362 of the slide 228. The gas stream impacts the surface of the evaporation liquid surface layer and moves the underlying reagent solution in a circular path on the tissue section. Preferably, the reagent solution is stirred on the surface of the biological sample by a vortex formed by applying two gas streams 356 and 347. Stream 356 is directed against a area 363 of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge 361. Stream 357, in a direction opposite to the direction of stream 356, is directed against an area 364 of the surface of the evaporation inhibiting liquid layer between the center of the evaporation inhibiting layer and the slide edge 362. Although this method is shown with respect to an evaporation liquid inhibitor covered reagent layer, it will be readily evident that it can be applied to gently stir any liquid layer overlying a fragile substance.

Fig. 18 is a schematic representational cross-sectional view of a slide following the rinse liquid, evaporation inhibitor and reagent application steps. Following the rinse stages (Stage A), the tissue section 371 mounted on slide 370 is covered with a thin residual aqueous layer 372. Following application of the evaporation inhibitor liquid (Stage B), the aqueous layer 372 and tissue section 371 is entirely covered by a layer 373 of the evaporation inhibitor liquid. Aqueous reagent 374, applied to the slide, flows under the evaporation inhibitor layer 373 to cover the tissue section. In the vortex mixing section (Stage C), air jets directed against the surface of the evaporation inhibitor liquid 373 move it and the reagent solution 374 thereunder in a swirling or stirring action on the

surface of the fragile tissue section. This gentle stirring achieves increased interaction of reagent with the tissue section while preserving the tissue from dehydration or other damage from the air jets.

Fig. 19 is a cross-sectional view of a rinse liquid container and associated heating components. The rinse liquid applied to the surface of the slides by rinse blocks 200, 202 and 204 should have a temperature above 40°C and is preferably about 45°C. The elevated temperature is critical for the immunochemical reactions. The rinse liquid is supplied by the hot water supply 44. The hot water supply 44 comprises an inner container of an inert material having a low coefficient of expansion such as a pyrex bottle 382 having a threaded neck 384 to which a cap 386 is attached by threads. The container 382 is surrounded by an insulating jacket 388 of suitable insulation material such as a fiberglass layer. Between the insulating jacket 388 and the bottle 382 is a heating jacket 390 with electrical power leads 392. A suitable heating jacket is a thick sheet of silastic rubber (polysiloxane) with embedded resistance heating coils having a combined heating value of about 180 watts. A conventional safety thermostat 394, connected to the elements of the heating jacket, is also provided between the insulating jacket 388 and bottle 382. The safety thermostat prevents the rinse liquid temperature from exceeding a preset value, preferably about 50°C. A thermistor temperature sensor 391 with leads 393 extends through the cap 386 into the upper zone of the bottle 382. An liquid inlet tube 394 extends through the cap 386 to the bottom of the neck 384, and an outlet tube 396 extends through the cap 386 to the bottom of the bottle 382.

This unique configuration provides a highly uniform liquid output temperature. The colder water entering through the inlet tube 394, being more dense than the heated liquid in the bottle, sinks downward past the heated container walls and is heated. The displaced liquid rises upward in the container. This stirring motion thoroughly mixes the liquid without the need for an agitator, producing a highly uniform outlet liquid temperature. Thermistor 391 constantly monitors the liquid temperature, providing a signal to the control system which is used to determine when the heating elements in jacket 390 should be energized.

Fig. 20 is a bottom, isometric view of a reagent container support tray 10. The reagent container tray 10 has feet 800, 801 and 802 which rest in respective matching recesses in the reagent carousel support 314 (Fig. 15) in only one position. This insures that the reagent tray and the reagent bottle receptors 11 are always positioned in predetermined orientation on the carousel support 314.

The feet 800, 801 and 802 also function as supporting feet when the reagent support is removed. Refrigeration of the reagents is often required during their storage. The reagent container tray 10, with the reagent bottles supported thereon, can be lifted from the carousel support 314 and placed in a refrigerator, supported by the feet 800, 801 and 802.

Indexing metal homing block 803 is mounted on the reagent container support 10 and rotates with the support. A conventional metal proximity detector (not shown) is mounted on the upper plate 8 at an position which places it adjacent the rotational path of the homing block. A change in electrical signal from the

proximity detector indicates that the metal homing block is in the 'home' position adjacent the block.

Fig. 21 is a fragmentary cross-sectional view taken along the line D-D in Fig. 11. Indexing block 229 is a metal block. Proximity sensor 610 is supported on the underside of plate 22 by bracket 611. The proximity sensor 610 emits an electrical signal through leads 612 which changes when the metal block 229 is positioned in the 'home' position immediately above the sensor.

The homing systems of the reagent carousel and slide support carousel operate in a similar manner. Presence of an indexing block adjacent the sensor produces a signal indicating that the carousel is in a "home" position, and provides a reference for subsequent indexed movements of the respective stepper motor drive and subsequent indexed movements of the respective carousel.

Fig. 22 is a schematic view of the pneumatic system of the automated immunostaining apparatus of this invention. The air supply for the system is supplied by air compressor 80 and air filter 82. The output line 400 from the air filter 82 is connected to the input port of air pressure regulator 86 where it is regulated to a constant output pressure of about 25 psi. Diaphragm pressure switch 402 communicates with the air pressure regulator 86 outlet line 403 through line 404. Diaphragm pressure switch 402 closes the system circuit breaker 406 when the pressure in line 404 is at least 22 psi. Failure of the air compressor and resulting drop in line pressure automatically deactivates the system.

The air output branch line 408 lead is connected by line 410 with tipper air cylinder three way control solenoid valve 412. When in an "open" position, solenoid valve 412 provides communication between input line and cylinder 278. This permits pressurized air to pass from

line 410 to air cylinder 278, thus pressing tipper 148 (Fig. 14) against the respective slide support tab 112 and tipping the slide support 206. When solenoid valve 412 returns to the vent position, the air cylinder communicates with atmosphere, permitting the air cylinder to return to its resting position. Tipper 148 then rises to its resting position, allowing the slide support to also return to its horizontal position. Three way solenoid valves 416 and 420 operate in an identical way, providing communication between the air inlet lines 414 and 418 and the respective air cylinders 280 and 282 when in the open position and actuating respective tippers 284 and 286. They also open communication between the air cylinders 280 and 282 and the atmosphere in the vent position, allowing the tippers to return to their elevated position.

Branch line 422 leads from line 408 to the reagent dispenser three way control solenoid valve 424. When energized to an "open" position, solenoid valve 424 permits pressurized air to pass from line 422 to air cylinder input line 300, causing rod 302 and foot 306 (Fig.15) to press the reagent dispenser bottle 12 downward, emitting a precise volume of reagent liquid. When solenoid valve 424 is in the vent position, the air cylinder 18 and the reagent bottle 12 return to their resting positions.

Branch line 426 leads from line 403 to branched lines 428 and 430. Branch line 428 leads to pressure regulator 38, providing an output pressure of 10 psi in output line 431. Three way solenoid valve 432, when in the open position, provides communication between air input line 431 to the evaporation inhibitor liquid reservoir container 434 through lines 436 and 438. It also delivers pressurized air to the rinse liquid supply

container 44 through line 440, rinse solution reservoir 441 and supply conduit 443. When solenoid valve is opened to atmosphere (vent position), air in line 436 and in containers 44 and 434 bled or vented to the atmosphere. This permits removal, opening or replacement of reservoir container 434, or opening or removal of supply container 441. The pressured air in containers 434 and 441 forces liquid through respective output conduits 442 and 443.

Conduit 442 leads to two way solenoid valve 446, which has an outlet conduit 448 leading to the evaporation inhibitor application block 212 and associated nozzles. When the solenoid 446 is opened, evaporation inhibitor liquid is emitted from nozzles 294 (Fig. 14 and 15) onto the surface of the respective slide 238.

Conduit 444 delivers pressurized rinse liquid from heated rinse liquid container 44 to branch conduits 450, 452 and 454 leading to conventional rinse liquid two way solenoid valves 460, 462 and 464. When the solenoid valves 460, 462 and 464 are opened, pressurized rinse liquid is delivered to the respective rinse blocks 200, 202 and 204 through supply conduits 260, 268 and 276. The pressurized rinse liquid is emitted by the rinse blocks onto the slides positioned in the respective station (Fig. 13).

Branch line 430 leads to pressure regulator 64, providing an output pressure of 15 psi in output conduit 466 leading to vortex mixer air control two way solenoid valve 468. When in the open position solenoid valve 468 delivers pressurized air to output conduit 470 connected thereto. Conduit 470 leads to branch lines 472 and 474 leading to vortex mixing blocks 222 and 224. The pressurized air is emitted by nozzles 351 and 355

(Fig. 17), stirring the reagent layer on the respective slides 234.

Fig. 23 is a schematic drawing of the 120 volt AC power distribution in the apparatus of this invention. The power circuit to power line filter 500 includes a main fuse 504 and main power switch 506. 120 Volt AC power to the air compressor 80 is provided by line 511 from the line fuse 510 in the I/O board 508. 120 Volt AC power to the air compressor cooling fan 514 is provided by line 513 from line fuse 512 in the I/O board 508. 120 Volt AC power to the electronics cooling fan 518 is provided by line 517 from line fuse 516 in the I/O board 508. 120 Volt AC power to the 24 volt DC power supply is provided by line 521 from line fuse 520 in the I/O board 508. 120 Volt AC power to the 5 volt/12 volt DC power supply 78 is provided by line 524 from line fuse 522 in the I/O board 508. 120 Volt AC power to the computer card rack 529 is provided by line 528 from line fuse 526 in the I/O board 508. 120 Volt AC power to slide heater fan relay 533 is provided by line 532 from line fuse 530 in the I/O board 508. 120 Volt AC power to the slide heater relays 537 is provided by line 536 from fuse 534 in the I/O board 508. 120 Volt AC power to the rinse fluid heater relay 541 is provided by line 540 from fuse 538.

Fig. 24 is a schematic drawing of the DC power distribution in the apparatus of this invention. 12 Volt DC logic power for printer 550 is provided by line 552 from the power supply 78. Similarly, 12 volt DC power for low slide temperature controller 68 is provided by line 554, 12 volt power for high slide temperature controller 70 is provided by line 556, and 12 volt power for rinse fluid temperature controller 66 is provided by line 558. 5 Volt DC laser power for the slide bar code

reader 231 is provided by line 560 from the power supply 78, and 5 volt power for the laser of reagent bar code reader 346 is provided by line 562. 5 Volt DC power to the liquid crystal display 34 is provided by line 564.

24 Volt DC power is provided to the upper motor controller 566 for the stepper motor 14 by line 568. 24 Volt DC power for the lower motor controller 570 for the stepper motor 48 is provided from power supply 76 by line 572.

The conventional card rack 529 has a separate 5 volt/12 volt power supply 576. 5 Volt DC logic power and 12 volt DC motor power is provided to the floppy disc drive by lines 574.

Fig. 25 is a schematic drawing of a first portion of the computer digital I/O system in the apparatus of this invention. The control system uses a series of standard optical relays, each of which are connected to close the line to ground in the power circuit for the respective component. The optical relays provide isolation.

Communication between the optical relays and the computer digital I/O board 580 is provided by lines 582. The two way solenoid valves 460, 462 and 464 controlling the rinse liquid flow from heated rinse supply 44 to the respective rinse blocks 200, 202 and 204 are energized to an open position and de-energized to a closed position by output signals from the computer digital I/O board 580 to the optical relays 584, 586 and 588. The two way solenoid valve 446 controlling the flow of evaporation control liquid from container 434 to the nozzle block 212 is energized to an open position or de-energized to a closed position by output signals from board 580 to optical relay 590.

The three way solenoid valves 412, 416 and 420 controlling air flow to the respective tipper air

cylinders 278, 280 and 282 are energized to an open position (causing air flow) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board 580 to respective optical relays 592, 594 and 596. The three way solenoid valve 424 controlling air flow to the micro delivery reagent dispenser control cylinder 300 is energized to an open position (causing air flow and reagent delivery) or de-energized to a closed position (venting cylinder air to the atmosphere) by output signals from computer I/O board 580 to respective optical relay 598. The two way solenoid valve 468 controlling air flow to the vortex air mixer blocks 220, 222 and 224 (Fig. 17) is energized to an open position (causing air flow to the mixer blocks) or de-energized to a closed position by output signals from computer I/O board 580 to respective optical relay 600.

The sound alarm 602 is activated to produce sound by an output signal from the computer I/O board 580 to optical relay 604. The sound alarm 602 can be activated to sound a 'beep' by keyboard key operation, by a longer 'beep' or double 'beep' at the completion of a run, and a sustained sound during a system malfunction, for example. The three way solenoid valve 432 controlling air flow to the rinse liquid and evaporation control liquid supply containers 44 and 434 (Fig. 22) is energized to an open position (causing air flow and pressurization of the supply containers) or de-energized to a closed position (venting cylinder air from the containers to the atmosphere) by output signals from computer I/O board 580 to respective optical relay 606.

The slide heat fan 56 speed is operated by pulse width modulation, that is, power pulses from the power relay 608. The fan 56 is energized by an output signal

to the power relay 608 from optical relay 610. The timed signal to the optical relay 610 is received from the computer I/O board 580. The pulse width and speed of the fan 56 is adjusted in response to heating requests from the high temperature slide controller 632 to increase the volume of heatng air delivered to the air distribution manifold 30.

The slide heater system control supplies separately controlled power to each of the resistance heating elements 331 and 332. Low temperature heating element 332 is energized by power relay 612 upon a signal from the low slide temperature controller 614. Thermistor 347 provides temperature information to the controller 614. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element 332 is turned on when operating heat is required, in response to a low temperature signal from the low temperature controller 614. It is turned off when the operating temperature is restored. The controller 614 also detects when the slide door switch 616 is closed. If the cabinet slide door is open, energy supply to the heating element 331 and 332 is interrupted. The heating cycle is initiated by a request for heat passed to the computer I/O board 580 through line 624 to the optical relay 622. The computer then responds with a heating power select heat signal received by controller 614 through line 620 from optical relay 618 in response to an output signal from the computer I/O board 580. A status signal for the slide door switch is received by the computer I/O board through line 628 and optical relay 626.

The high temperature heating element 331 is energized by power relay 630 upon a signal from the high slide temperature controller 632, in response to a power

command signal through optical relay 634 and line 636 from the computer digital I/O board 580. During the operation of the apparatus at the lower temperatures required for the immunohistological processes, the power to the heating element 331 is turned on only during an initial warm-up cycle. During the warm-up cycle, heat energy is requested from the I/O board 580 through line 638 and optical relay 640.

When the apparatus is operated at the higher temperatures required for *in situ* hybridization, the heating elements are energized in a different control sequence by the controllers 614 and 632. As with the low temperature operation, both heating elements 331 and 332 are energized during the warm-up cycle. However, in the high temperature operating mode, the low temperature heating element 332 is continuously energized, and energy is supplied intermittently to the heating element 331. In the high temperature mode, therefore, the optical relay 634 receives a power command signal from the I/O output board 580 when the high temperature controller 632 signals that more heat is required. In addition to the heater controls described above, an additional thermostat is provided in the heater circuit which turns the heater off if the heater temperature reaches 160°C, for example if the fan 56 fails.

The rinse liquid heating system resistance heater 390 (Fig. 19) is energized through power relay 642 upon a signal from rinse fluid controller 644. Thermistor 391 monitors the rinse fluid temperature, and the controller 644 provides a signal indicating whether or not further heat energy is required. A heat request signal for heating liquid is received by the computer I/O board through line 646 and optical relay 648. The computer

responds with a heat select signal from the I/O board 680 through relay 650 and line 652.

Fig. 26 is a schematic drawing of a second portion of the computer digital I/O system in the apparatus of this invention. The computer digital I/O board 580 receives a signal indicating closure of the air pressure switch 402 (Fig. 22) through line 670 and optical relay 672. The computer digital I/O board 580 receives a home signal from the reagent carousel metal proximity home sensor through line 676 and optical relay 674 when the metal block 803 and the reagent carousel 10 are in the home position. The computer digital I/O board 580 receives a home signal from the slide support metal proximity home sensor 610 through line 680 and optical relay 678 when the metal block 229 and the slide support carousel 24 are in the home position.

The reagent carousel stepper motor 14 is operated by reagent carousel stepper motor controller 690 in response to commands received from the computer digital I/O board 580. Command signals for steps (motor operation) are received through line 692, and command signals for the direction of operation are received through line 694. The stepper motor has a high and low torque operating mode, the low torque mode being effected by switching a resistor into the control circuit. The high torque mode is used to move the motor through the number of steps required to place a selected reagent bottle in the reagent delivery station. The low torque mode is used as a brake to hold the reagent bottle carousel in a position. The low or high torque command signal is received by the reagent carousel stepper motor controller 690 through line 698 and optical relay 696.

The slide support carousel stepper motor 48 is operated by slide support carousel stepper motor

controller 700 in response to commands received from the computer digital I/O board 580. Command signals for steps (motor operation) are received through line 702, and command signals for the direction of operation are received through line 704. This stepper motor also has a high and low torque operating mode, activated in the same way and having the same functions as the reagent carousel stepper motor operating modes. The high torque mode is used to move the motor through the number of steps required to place a selected slide in a selected treatment zone. The low or high torque command signal is received by the slide support carousel stepper motor controller 700 through line 708 and optical relay 706. When the door switch 616 shows an open door status, the step command signals to the stepper motors 14 and 48 are prevented. If the door switch 616 is opened during a biological processing run, any incomplete stepper motor sequence is permitted to reach completion before further step command signals are blocked.

The keyboard 710 is a conventional pressure sensitive keyboard. The switches 720-726, 730-736, 740-746 and 750-756 are closed by manual pressure applied to the surface of an impermeable flexible plastic layer over the switches. The switches are isolated and protected under the plastic layer and are not fouled by moisture or debris from the laboratory or operator.

In operation input lines 711, 712, 714 and 716 are each sequentially energized for a brief period by the computer digital I/O board 580, and the lines 718, 728, 738 and 740 are each sequentially polled during this brief period. If line 718 polls positive while line 716 is energized, closure of switch 720 is indicated. In a similar manner, closure of switch 722 is indicated by a positive poll of line 718 when line 714 is energized,

closure of switch 724 is indicated by a positive poll of line 718 when line 712 is energized, closure of switch 726 is indicated by a positive poll of line 718 when line 711 is energized, and the like.

Fig. 27 is schematic drawing of the computer serial and floppy disk I/O system in the apparatus of this invention. The computer RS-232 I/O port 770 sends polling signal to the slide barcode reader 231 and receives signals indicating bar code information read through line 772. Similarly, the computer RS-232 I/O port 770 sends polling signal to the reagent carousel barcode reader 346 and receives signals indicating barcode information read through line 774. Signals to the liquid crystal display 34 are sent through line 776 from the RS-232 I/O port 770. The computer RS-232 I/O port 770 receives an availability polling signal from the printer 550 and sends digital data to printer 550 through line 778.

Immunohistological methods for which the apparatus of this invention are particularly suitable are described in concurrently filed, commonly assigned patent application Serial No. _____, filed March 2, 1990 (Attorney Docket No. 193.0007), the entire contents of which are hereby incorporated by reference. A typical immunohistological method, as carried out with the apparatus of this invention includes the following steps.

1) Preparing the slides, including applying a bar code to the slide indicating the immunohistological process to be used with the sample, and manually rinsing and applying evaporation inhibiting liquid to the tissue sample surface before placement in the apparatus to prevent dehydration of the sample.
2) Inserting a batch of slides in the apparatus, mounting each slide in a slide support.

3) Closing the apparatus and beginning the treatment processing. The apparatus heating system is in the warm-up mode until the heating air temperature reaches the desired level.

4) A slide is rinsed in the first rinse station (Fig. 11-14) in seven rinse cycles. Each cycle includes applying a 500 μL pulse of rinse liquid followed by tipping the slide support to effect draining. This sequence can be repeated for seven rinse cycles as the slide is moved to and pauses in each of the second and third rinse stations, for a total of twenty-one rinse cycles, for example. The slide then is treated in a seven second stay in the evaporation inhibitor and reagent solution application station (Fig. 11, 14 and 15). An initial quantity of 500 μL of an evaporation inhibiting liquid such as dodecane is applied to the slide surface. Then 200 μL of reagent solution is applied to the slide.

As each slide poises in the reagent application zone, the appropriate reagent container is moved by the reagent carousel to the reagent application station, and a metered volume of reagent is applied to the slide. In being applied to the slide, the reagent liquid is applied to the uppermost surface (the evaporation liquid layer). It then passes through the evaporation inhibiting liquid layer to the underlying aqueous layer, a procedure which would not be possible with a conventional solid glass coverslip.

6) The slide is then passed to each of the vortex mixing stations (Fig. 11, 14, 16 and 17). Here vortex jets stir the reagent on the slide surface under the file of evaporation inhibiting liquid.

This procedure would not be possible with a conventional solid glass coverslip.

7) The slide is then carried by the carousel, pausing as each slide support is sequenced through the same steps, until it returns to the initial rinse station, where the cycle is repeated. The reaction between the reagent and the tissue sample continues during this period, and slides in each of the following slide supports is subjected to the same sequence of rinse, application of evaporation inhibitor, application of reagent, stirring, and incubation.

8) In a typical immunohistological process using a four phase process with a peroxidase enzyme antibody label, a sequence total of five different reagents are applied as the tissue sample is passed five times through the reagent application zone. In such a process, the first reagent is a hydrogen peroxide solution required to eliminate endogenous peroxidase activity in the tissue sample. The second reagent is a primary antibody which binds selectively with an specific epitope for which the sample is being tested. The third reagent is a biotin labeled secondary antibody which binds preferentially with the primary antibody remaining on the sample following the preceding incubation and rinsing. The fourth reagent is avidin labeled with an enzyme such as a peroxidase enzyme, the avidin binding with the biotin label remaining on the sample following the preceding incubation and rinsing. The fifth reagent is a substrate solution which is converted by the peroxidase enzyme to form a detectable label such as a fluorophore or chromophore at the site of any primary antibody binding with the sample.

9) Following the conclusion of the substrate solution treatment and incubation, the slide typically is removed from the carousel, coverslipped with a glass coverslip and examined to determine the extent of primary antibody binding with the tissue sample.

WE CLAIM:

1. An automated biological reaction apparatus having a reagent support carousel with a plurality of reagent container supports thereon, homing and indexing means associated with the support carousel for identifying the position of each reagent container support with reference to a home position during the operation of the apparatus, and drive means engaging the carousel for rotating the carousel and positioning a preselected reagent container support in a reagent supply zone.

2. An automated biological reaction apparatus of Claim 1 wherein the reagent support carousel is rotatably mounted on a reagent carousel support and the homing and indexing means comprises a proximity detection means and an object detectably by the proximity detection means when the proximity detection means and said object are in close proximity, one of said object and said proximity detection means being mounted on the reagent support carousel, and the other of the object and said proximity detection means being mounted on the reagent carousel support in a position adjacent the path of the other.

3. An automated biological reaction apparatus of Claim 2 wherein said object is metallic and mounted on the reagent support carousel and the proximity detector is a metal proximity detector mounted on the reagent carousel support.

4. An automated biological reaction apparatus of Claim 1 wherein the reagent support carousel is rotatably mounted on a reagent carousel support, the reagent support carousel has a bar code zone, and the homing and indexing means comprises a bar code reader mounted on the reagent carousel support in a

position to read a bar code on a reagent bottle positioned in the bar code zone, whereby a bar code identifying the contents of a reagent container in the respective reagent container support can be read with reference to said home position by the bar code reader, and the reagent container containing said identified reagent can be automatically positioned in the reagent supply zone.

5. An automated biological reaction apparatus of Claim 1 including a reagent delivery actuator means positioned for engaging a reagent container positioned in the reagent delivery zone and initiating delivery of a predetermined volume of reagent from the reagent container.

6. An automated biological reaction apparatus of Claim 1 wherein the drive means comprises a stepper motor having a rotational mode for rotating the reagent carousel and a braking mode resisting rotation of the reagent carousel.

7. An automated biological reaction apparatus of Claim 1 wherein the reagent support carousel comprises a reagent support tray removably supported by a reagent tray support, the reagent support tray has indexing support feet on the underside thereof, the reagent tray support has receptors for the indexing support feet in the upper surface thereof, whereby the reagent support tray can be removed from the reagent tray support for reloading or refrigerated storage and can be replaced on the reagent support tray in the same indexed position.

8. A slide support for an automated biological reaction apparatus comprising a slide support plate having a distal end, a proximal end, and a slide support surface, the distal end having raised terminal and

lateral distal guide tabs with guide tab termini, the proximal end having first and second lateral guides with opposed surfaces for engaging the lateral edges of a slide, the distance between the slide support surface and the guide tab termini being less than a microscope slide thickness.

9. A slide support of Claim 8 wherein the support plate comprises a distal support section at the distal end and a proximal support section at the proximal end, the proximal support section comprising an inflexible support and a flexible arm with opposed lateral edges, and the distance between the slide engaging surfaces is less than a microscope slide width, whereby the slide engaging surfaces apply a positive pressure against the edges of a slide engaged therewith.

10. A slide support of Claim 9 wherein the distance between the slide engaging surfaces is from 20 to 24 mm.

11. A slide support of Claim 8 including a pivot support with a pivot axis, wherein the slide support plate is pivotally mounted on the pivot support for rotation around the pivot axis from a horizontal position to a slide draining position.

12. A slide support of Claim 11 wherein the pivot axis is defined by a pivot rod and a pivot rod receptor in sliding engagement therewith, one of the pivot rod and the pivot rod receptor being attached to or integral with the slide support and the other of the pivot rod and pivot rod receptor being attached to or integral with the pivot support.

13. A slide support of Claim 12 wherein the pivot axis is defined by two pivot rods and pivot rod receptors.

14. A slide support of Claim 11 wherein the slide support surface slopes downward from the proximal end to the distal end, the plane of the slide support surface forming an angle with the pivot axis of from 0.3 to 1°.

15. A slide support of Claim 11 wherein the slide support includes a lateral tilt cam surface for engagement by a tilt actuator.

16. A slide support of Claim 11 including a rotational bias means for retaining the support surface in the substantially horizontal position when the tilt cam surface is not engaged by a tilt actuator.

17. A slide support of Claim 16 wherein the rotational bias means is a spring.

18. A slide support of Claim 11 wherein the pivot support has a pivot stop means positioned to abut a surface of the slide support for stopping pivotal rotation of the slide support when it has been pivoted to the slide draining position.

19. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, homing and indexing means associated with the slide support carousel for identifying when the slide carousel is in a home position during the operation of the apparatus, and drive means engaging the carousel for rotating the carousel and positioning a slide support in a reagent delivery zone.

20. An automated biological reaction apparatus of Claim 19 wherein the slide support carousel is rotatably mounted on a slide carousel support, and the homing and indexing means comprises a proximity detection means and an object detectable by the proximity detection means when the proximity detection means

and said object are in close proximity, one of said object and said proximity detection means being mounted on the slide support carousel, and the other of the object and said proximity detection means being mounted on the slide carousel support in a position adjacent the path of the other.

21. An automated biological reaction apparatus of Claim 20 wherein said object is metallic and mounted on the slide support carousel and the proximity detector is a metal proximity detector mounted on the slide carousel support.

22. An automated biological reaction apparatus of Claim 19 wherein the slide support carousel is rotatably mounted on a slide carousel support, the slide support carousel has a bar code zone, and the homing and indexing means comprises a bar code reader mounted on the slide carousel support in a position to read a bar code on a slide positioned in the bar code zone.

23. An automated biological reaction apparatus of Claim 19 wherein the drive means comprises a stepper motor having a rotational mode for rotating the reagent carousel and a braking mode resisting rotation of the reagent carousel.

24. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, and drive means engaging the carousel for rotating the carousel and positioning a preselected slide support in a reagent delivery zone, heating means positioned for heating air and passing the heated air over the slide supports, said heating means comprising a wall means around the slide support carousel defining a heating chamber, air distribution manifold means

having a plurality of heated air outlet ports positioned above the slide supports for distributing heated air over the upper surfaces of the slide supports, and air heater means.

25. An automated biological reaction apparatus of Claim 24 wherein the air heater means comprises an air supply chamber communicating with the air distribution manifold, start-up and operational heating means positioned in the path of air passing from the air supply chamber to the air distribution manifold, the start-up heating means comprising means for heating air until the heating chamber has reached an operational temperature, and the operational heating means comprising means for heating air until the heating chamber has reached said operational temperature and for intermittently heating air thereafter to maintain the heating chamber at an operational temperature.

26. An automated biological reaction apparatus of Claim 25 wherein the air heater means includes a fan positioned to force air into the air distribution manifold through the air supply chamber, said fan including air temperature responsive means for increasing the rotational speed of said fan when the air temperature entering the air distribution manifold falls below a desired operational temperature.

27. An automated biological reaction apparatus of Claim 25 including a temperature sensing means positioned in the path of heated air entering the air distribution manifold for detecting the temperature of said heated air.

28. An automated biological reaction apparatus of Claim 27 wherein the temperature sensing means is a

thermistor encased in a heat sensitivity reducing jacket.

29. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, drive means engaging the carousel for rotating the carousel and positioning a slide support in a reagent delivery zone, and a rinse station, a rinse solution application means positioned adjacent the rinse station, the rinse solution application means comprising at least one nozzle positioned for directing a stream of rinse liquid onto a rinse solution impact zone of a slide support.

30. An automated biological reaction apparatus of Claim 29 wherein each slide support has a distal end, a proximal end, and a slide support surface, the distal end having raised terminal and lateral distal guide tabs with guide tab termini, the proximal end having first and second lateral guides with opposed surfaces for engaging the lateral edges of a slide, the distance between the slide support surface and the guide tab termini being less than a microscope slide thickness.

31. An automated biological reaction apparatus of Claim 30 wherein the slide support has a distal support section at the distal end and a proximal support section at the proximal end, the proximal support section comprising an inflexible support and a flexible arm with opposed lateral edges, and the distance between the slide engaging surfaces is less than a microscope slide width, whereby the slide engaging surfaces apply a positive pressure against the edges of a slide engaged therewith.

32. An automated biological reaction apparatus of Claim 31 wherein the distance between the slide engaging surfaces is from 20 to 24 mm.

33. An automated biological reaction apparatus of Claim 29, including a slide pivot support with a pivot axis, and wherein the slide support plate is pivotally mounted on the pivot support for rotation around the pivot axis from a horizontal position to a slide draining position.

34. An automated biological reaction apparatus of Claim 33 wherein the slide support includes a lateral tilt cam surface for engagement by the tilt actuator, and the apparatus includes a tilt actuator means positioned to applying pressure against the tilt cam surface and causing the slide support to move from a substantially horizontal position to a drain position.

35. An automated biological reaction apparatus of Claim 34 including a rotational bias means for retaining the slide support surface in the substantially horizontal position when the tilt cam surface is not engaged by a tilt actuator.

36. An automated biological reaction apparatus of Claim 35 wherein the rotational bias means is a spring.

37. An automated biological reaction apparatus of Claim 36 wherein the pivot support has a pivot stop means positioned to abut a surface of the slide support for stopping pivotal rotation of the slide support when it has been pivoted to the slide draining position.

38. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, drive means engaging the carousel for rotating the

carousel and positioning a slide support in a reagent delivery zone and a evaporation inhibiting liquid application station, evaporation inhibiting liquid application means positioned adjacent the application station, the evaporation inhibiting liquid application means comprising at least one nozzle positioned for directing a stream of evaporation inhibiting liquid onto a preselected evaporation inhibiting liquid impact zone of a slide support.

39. An automated biological reaction apparatus of Claim 38 wherein the evaporation inhibiting liquid application station is in the reagent delivery zone.

40. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, drive means engaging the carousel for rotating the carousel and positioning a slide support in a reagent delivery zone, and a reagent support carousel with a plurality of reagent container supports thereon, homing and indexing means associated with the support carousel for identifying the position of each reagent container support with reference to a home position during the operation of the apparatus, and drive means engaging the carousel for rotating the carousel and positioning a preselected reagent container support in a reagent supply zone.

41. An automated biological reaction apparatus of Claim 40 wherein the reagent support carousel is rotatably mounted on a reagent carousel support and the homing and indexing means comprises a proximity detection means and an object detectably by the proximity detection means when the proximity detection means

and said object are in close proximity, one of said object and said proximity detection means being mounted on the reagent support carousel, and the other of the object and said proximity detection means being mounted on the reagent carousel support in a position adjacent the path of the other.

42. An automated biological reaction apparatus of Claim 41 wherein said object is metallic and mounted on the reagent support carousel and the proximity detector is a metal proximity detector mounted on the reagent carousel support.

43. An automated biological reaction apparatus of Claim 40 wherein the reagent support carousel is rotatably mounted on a reagent carousel support, the reagent support carousel has a bar code zone, and the homing and indexing means comprises a bar code reader mounted on the reagent carousel support in a position to read a bar code on a reagent bottle positioned in the bar code zone, whereby a bar code identifying the contents of a reagent container in the respective reagent container support can be read with reference to said home position by the bar code reader, and the reagent container containing said identified reagent can be automatically positioned in the reagent supply zone.

44. An automated biological reaction apparatus of Claim 40 comprising a reagent delivery actuator means positioned for engaging a reagent container positioned in the reagent delivery zone and initiating delivery of a predetermined volume of reagent from the reagent container to a preselected reagent impact zone of a slide support in the reagent delivery zone.

45. An automated biological reaction apparatus having a slide support carousel with a plurality of slide supports mounted thereon in a circular array, drive means engaging the carousel for rotating the carousel and positioning a slide support in a vortex agitation zone, a vortex agitation means positioned adjacent the vortex agitation zone and having a nozzle means for directing air at the vortex agitation zone.

46. An automated biological reaction apparatus of Claim 45 wherein the vortex agitation means comprises a nozzle means for applying at least one gas stream to an off-center area of the surface of liquid on a slide in the vortex agitation zone.

47. An automated biological reaction apparatus of Claim 46 wherein the vortex agitation means comprises a first nozzle means adjacent to the distal end of a slide support in the vortex agitation zone for directing a first gas stream to a first off-center area of the surface of the liquid on a slide in the vortex agitation zone, and a second nozzle means adjacent to the proximal end of a slide support in the vortex agitation zone for directing a second gas stream to a second off-center area of the surface of the liquid on a slide in the vortex agitation zone, the first and second gas streams being in opposite directions and the first and second off-center areas being on opposite sides of the center of the surface of a liquid on a slide in the vortex agitation zone.

48. A heated liquid supply comprising a container having a top opening and a cap means for closing the opening, a heating jacket on at least a portion of the outer surface of the liquid container, insulation means surrounding the outer surface of

the liquid container and the heating jacket, a temperature sensing means, a liquid input conduit and a liquid output conduit extending into the liquid container through the cap means, the liquid input conduit having an outlet adjacent the top of the liquid container and the liquid output conduit having an inlet adjacent the bottom of the liquid container, and a power supply means connected to the temperature sensing means for energizing the heating jacket when the temperature of liquid in the container is below a lower predetermined level and for de-energizing the heating jacket when the temperature of liquid in the container is above an upper predetermined level.

49. A heated liquid supply of Claim 48 including a safety thermostat connected to the heating jacket for terminating flow of power to the heating jacket if the temperature of the container exceeds a predetermined safety limit above the upper predetermined level.

50. An improved biochemical method with increased sample dehydration protection comprising applying a biochemical reagent to the surface of a solid biological sample on a planar support surface, the improvement comprising

a) covering the sample with an aqueous surface layer by applying an aqueous solution to the planar support surface adjacent the biological sample;

b) covering the aqueous surface layer with an evaporation inhibiting liquid layer by applying the evaporation inhibiting liquid to the planar support surface adjacent the biological sample in an amount sufficient to form a continuous

layer of evaporation inhibiting liquid over the sample, the evaporation inhibiting liquid being substantially water-insoluble, substantially water-immiscible and substantially non-viscous; having a specific gravity less than water, and a boiling point above 100°C; and being devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample; and

c) and treating the biological sample with an aqueous reagent solution by applying the reagent solution to the planar support surface adjacent the biological sample, whereby the reagent solution flows to the biological sample under the evaporation inhibiting liquid layer.

51. An improved biochemical method Claim 50 wherein the evaporation inhibiting liquid is a substantially saturated alkane or cycloalkane having from 8 to 18 carbon atoms.

52. An improved method for mixing a reagent solution layer on a sample mounted on a planar support surface comprising stirring the reagent solution by applying at least one gas stream to an area of the reagent solution layer between the center of the reagent solution layer and the edge of the planar surface, the axis of the gas stream forming an acute angle with the planar support surface.

53. An improved method of Claim 52 wherein the reagent solution layer is covered by a layer of an evaporation inhibiting liquid, the evaporation inhibiting liquid being substantially water-insoluble, substantially water-immiscible and substantially non-viscous; having a specific gravity less than water, and a boiling point above 100°C;

and being devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample.

54. An improved method of Claim 52 for mixing a reagent solution layer on a sample comprising applying two gas streams to areas of the reagent solution layer to areas between the center of the reagent solution area and the edge of the planar support surface, the first gas stream being directed against a first reagent solution area and the second gas stream being directed against a second reagent solution area, the first and second gas streams being in opposite directions, and the first and second reagent solution areas being on opposite sides of the center of the reagent solution area.

55. An improved method of Claim 54 wherein the reagent solution layer is covered by a layer of an evaporation inhibiting liquid, the evaporation inhibiting liquid being substantially water-insoluble, substantially water-immiscible and substantially non-viscous; having a specific gravity less than water, and a boiling point above 100°C; and being devoid of chemical characteristics which would significantly interfere with biochemical reactions carried out on the sample.

ABSTRACT OF THE DISCLOSURE

An automated immunostaining apparatus having a reagent application zone and a reagent supply zone. The apparatus has a carousel slide support supporting a plurality of slide supports thereon, and drive means engaging the carousel slide support for consecutively positioning each of a plurality of slide supports in the reagent application zone. The apparatus also has a carousel reagent support having a plurality of reagent container supports thereon, and drive means engaging the carousel for rotating the carousel and positioning a preselected reagent container support in the reagent supply zone. The apparatus also has a reagent delivery actuator means positioned for engaging a reagent container positioned on a container support in the reagent delivery zone and initiating reagent delivery from the reagent container to a slide supported on a slide support in the reagent receiving zone.

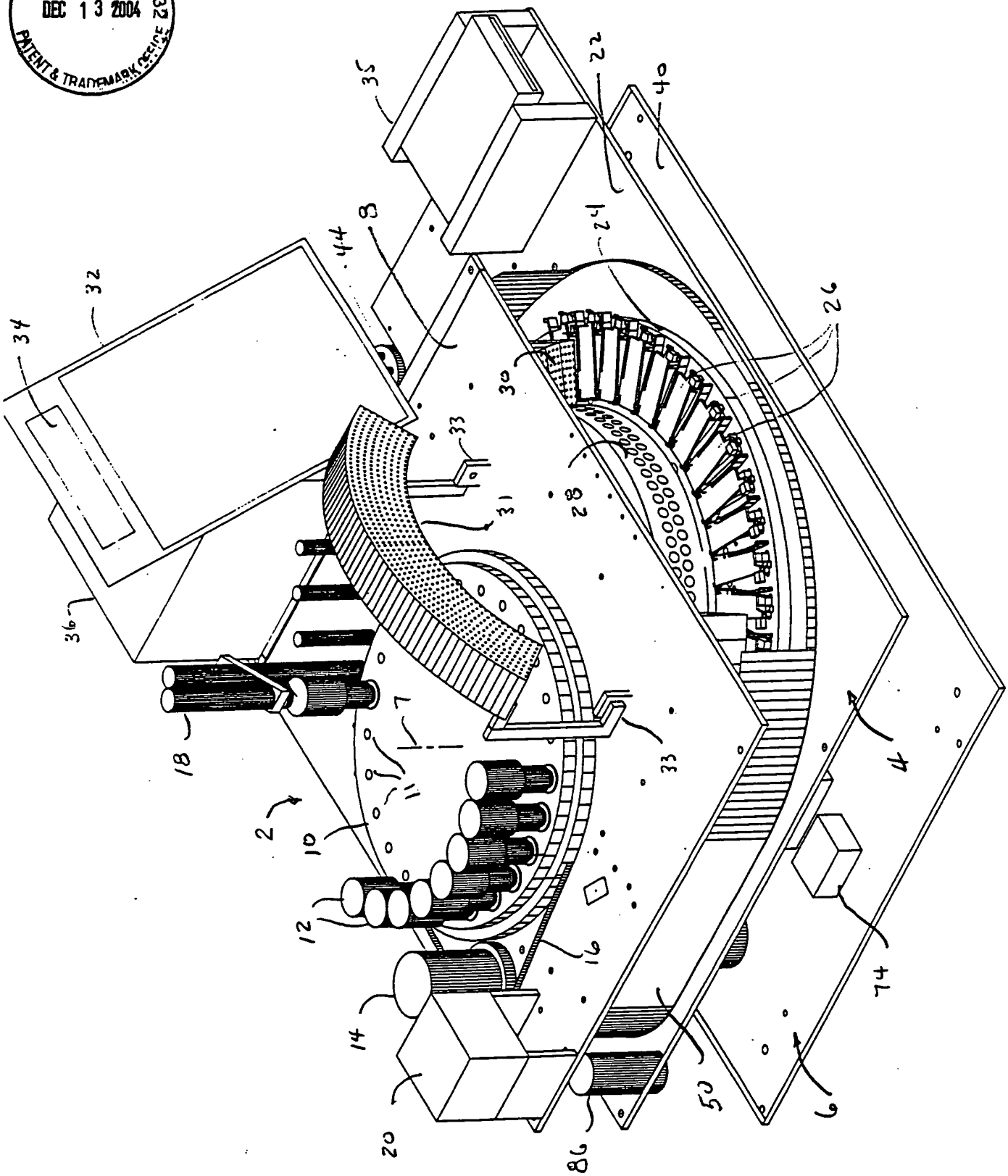

FIG. 1

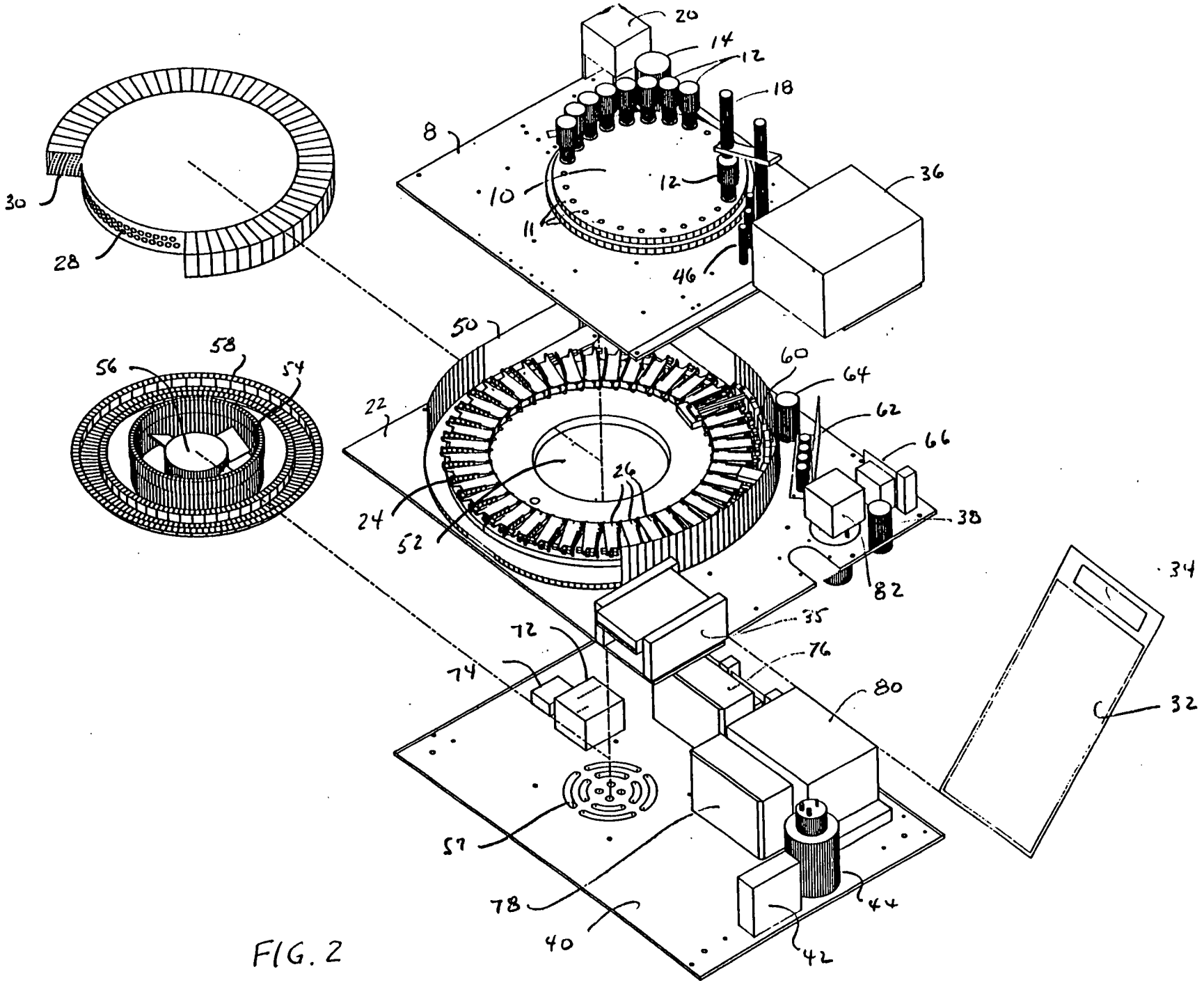
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FIG. 2

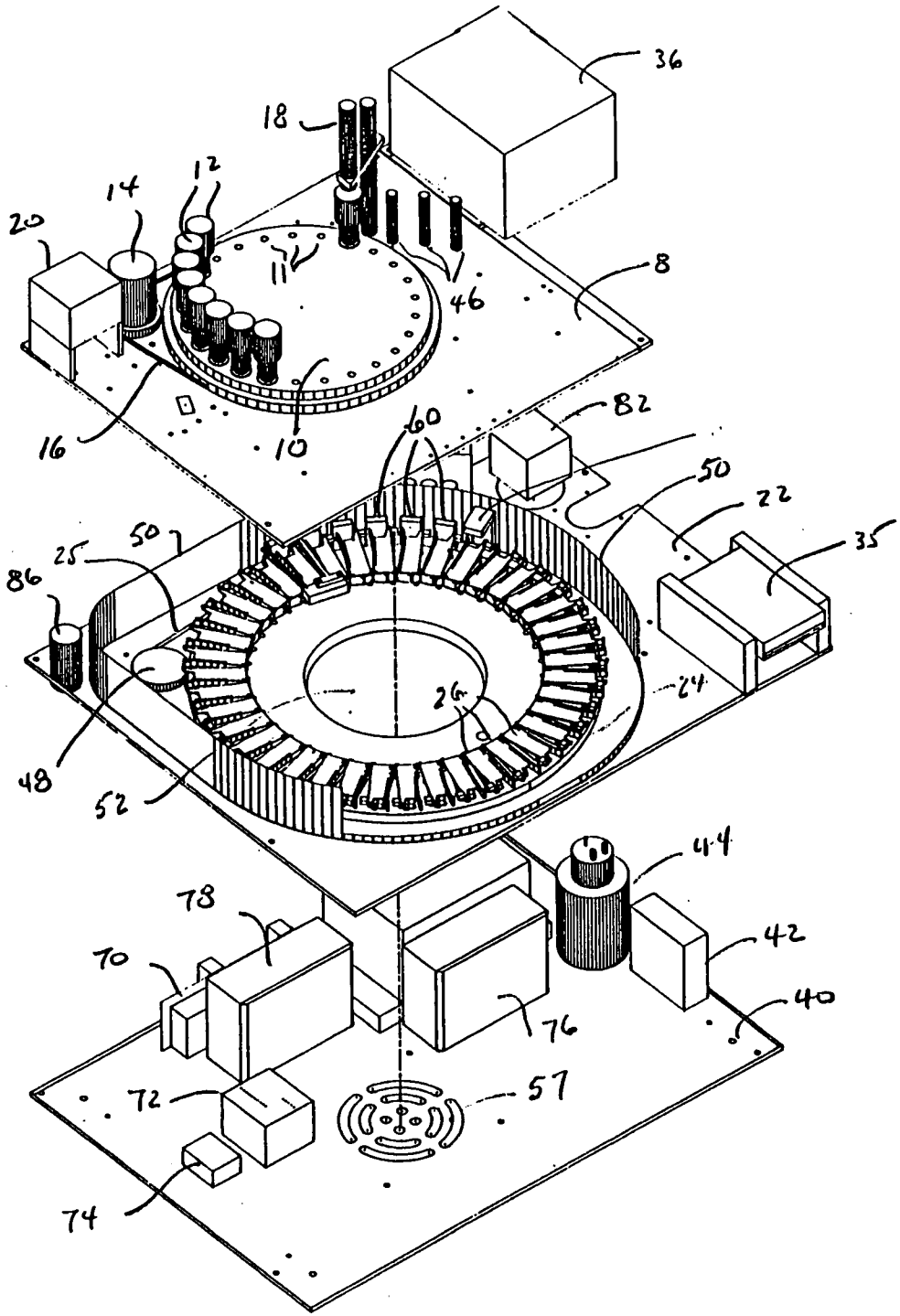
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FIG. 3

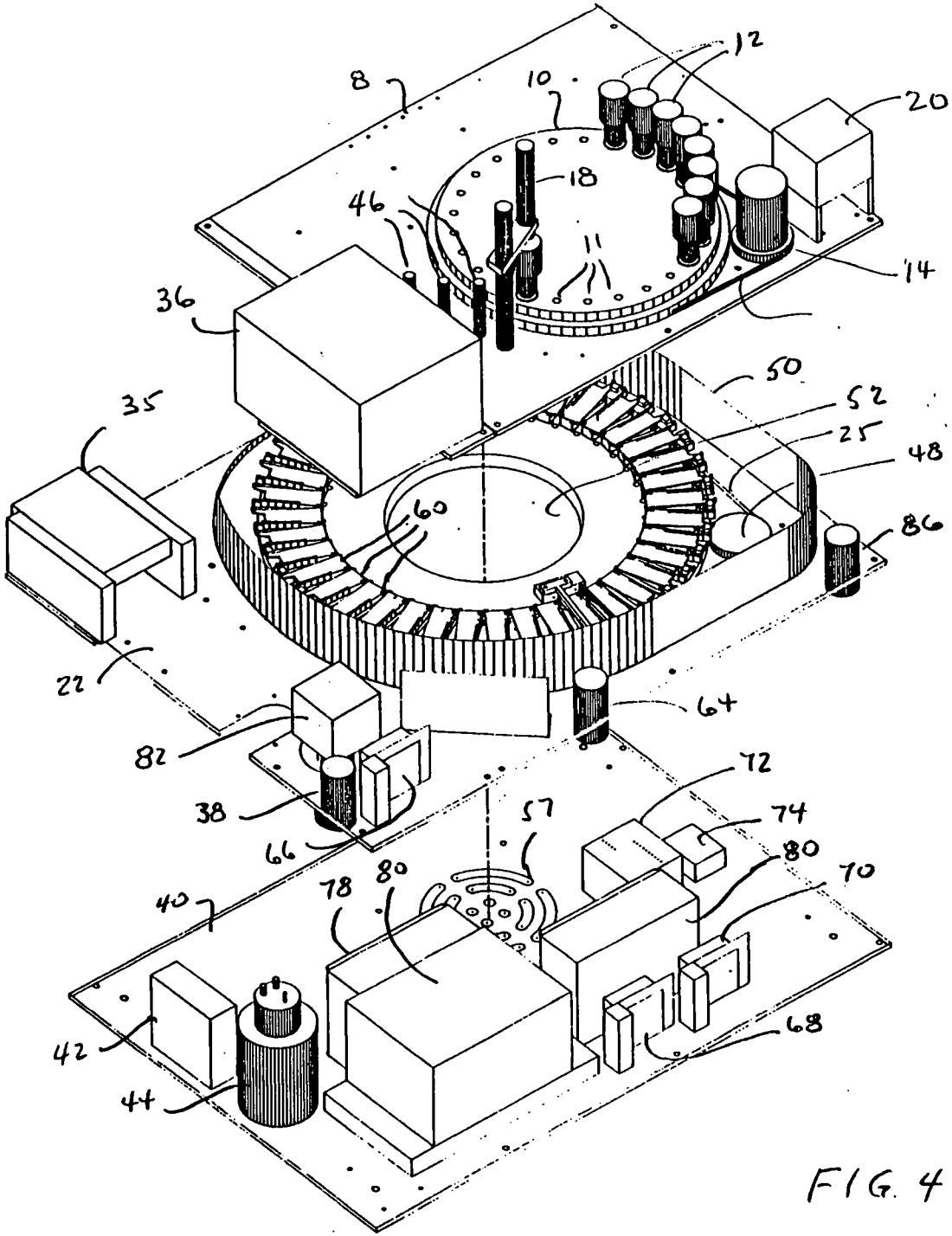
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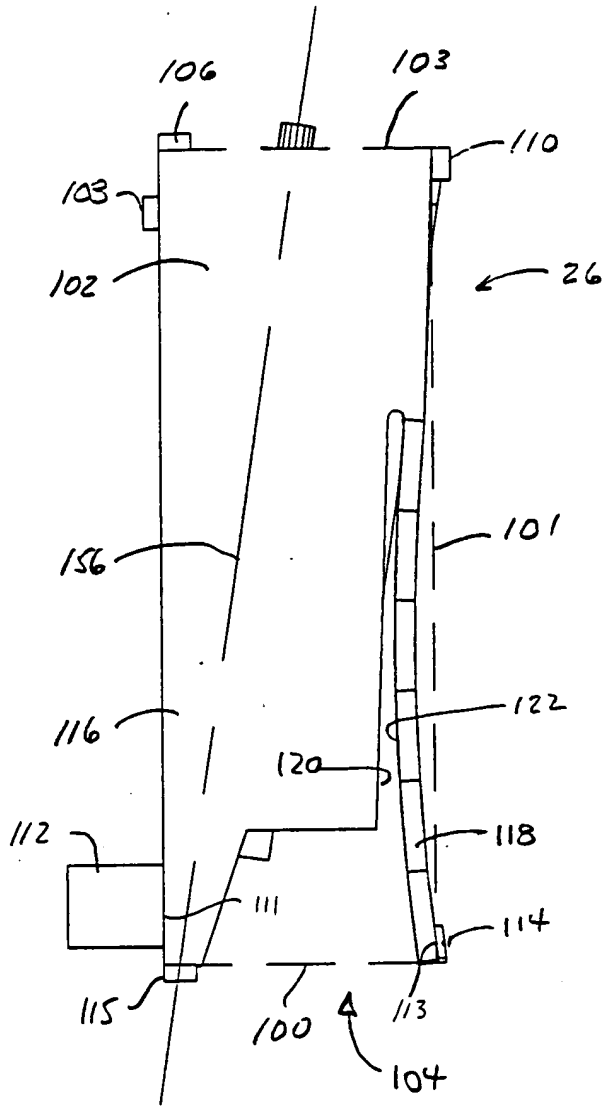
FIG. 4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115
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113
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FIG. 5

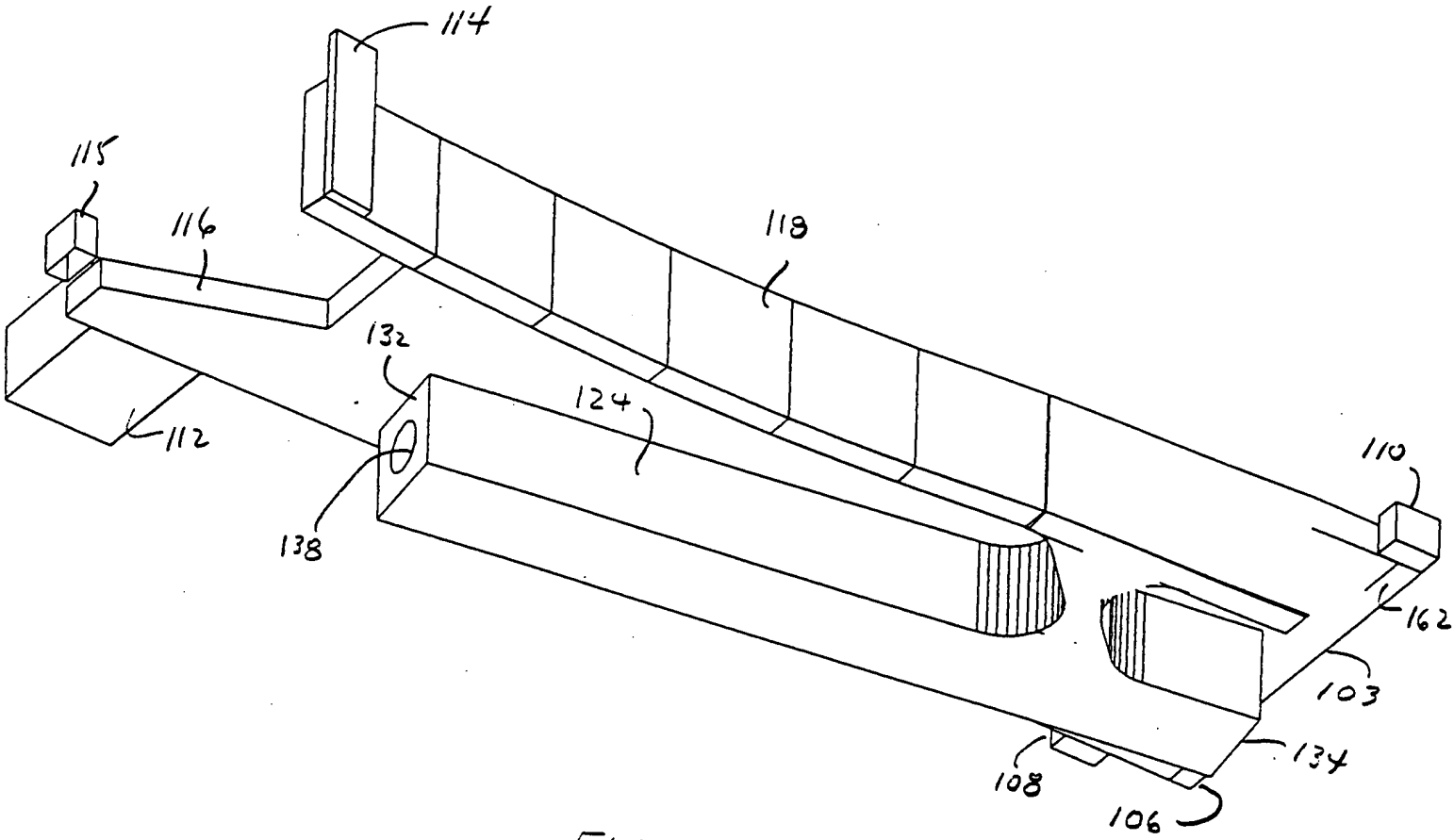

FIG. 6

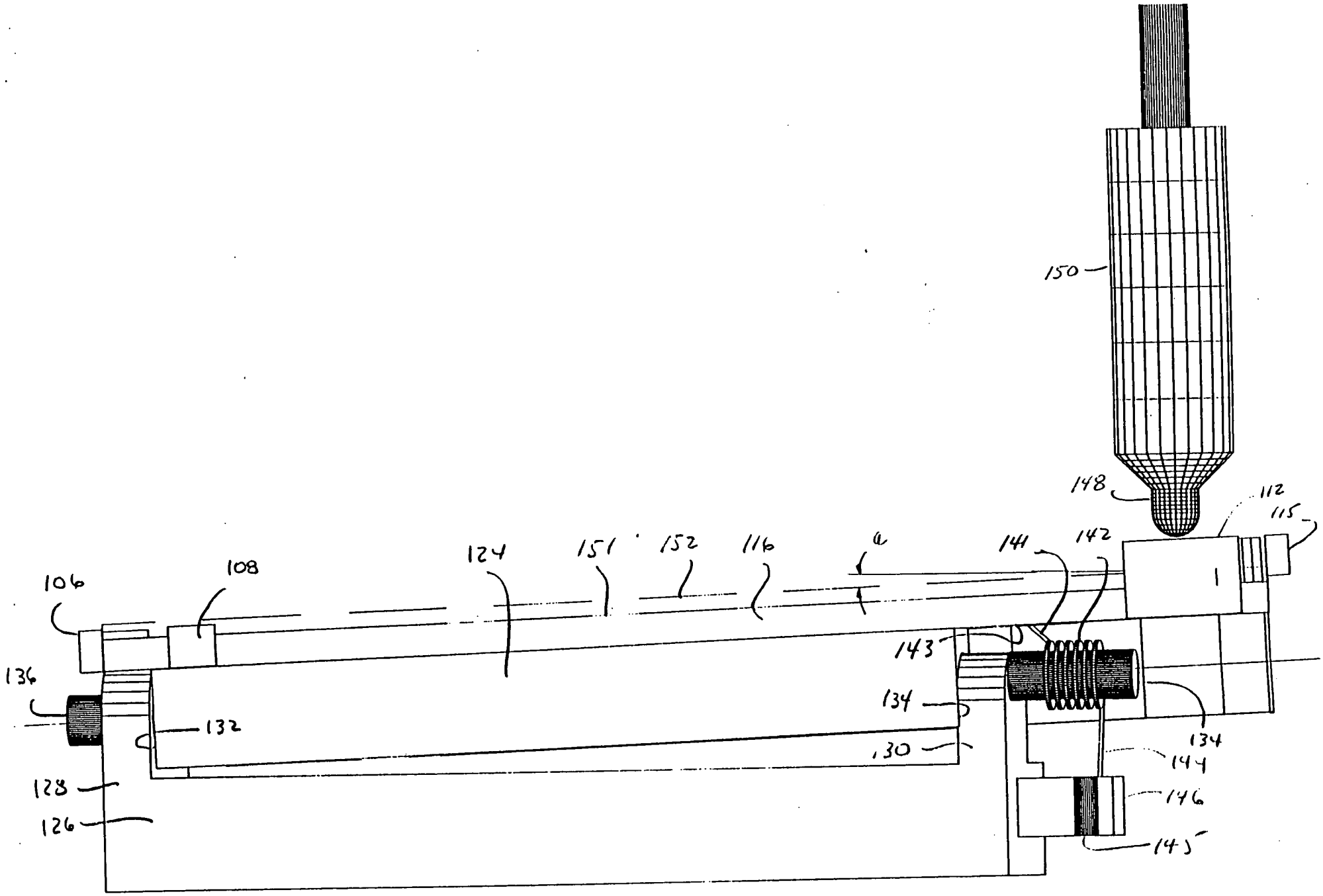
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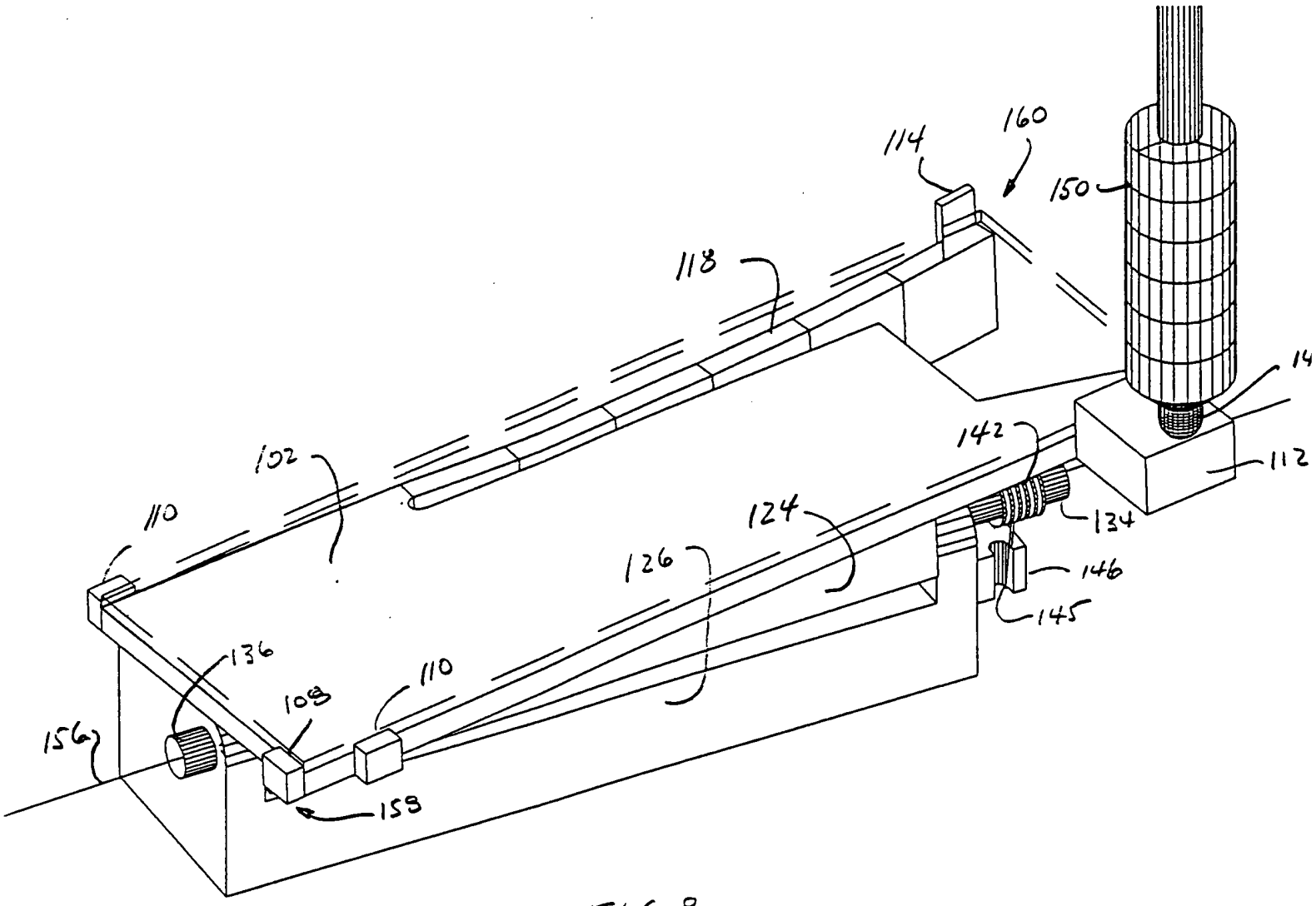
FIG. 7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146
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136
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156
159

FIG. 8

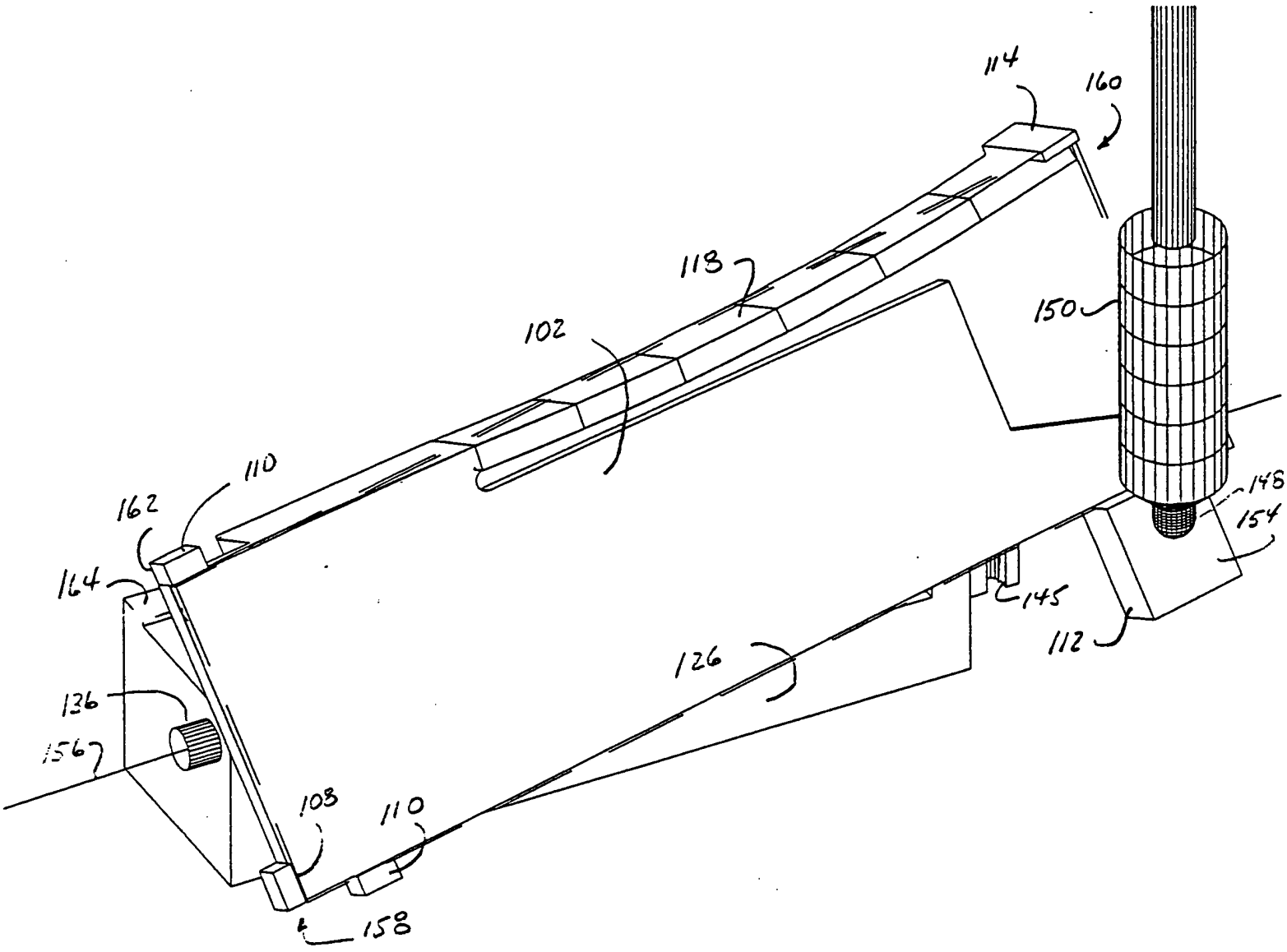
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FIG. 9

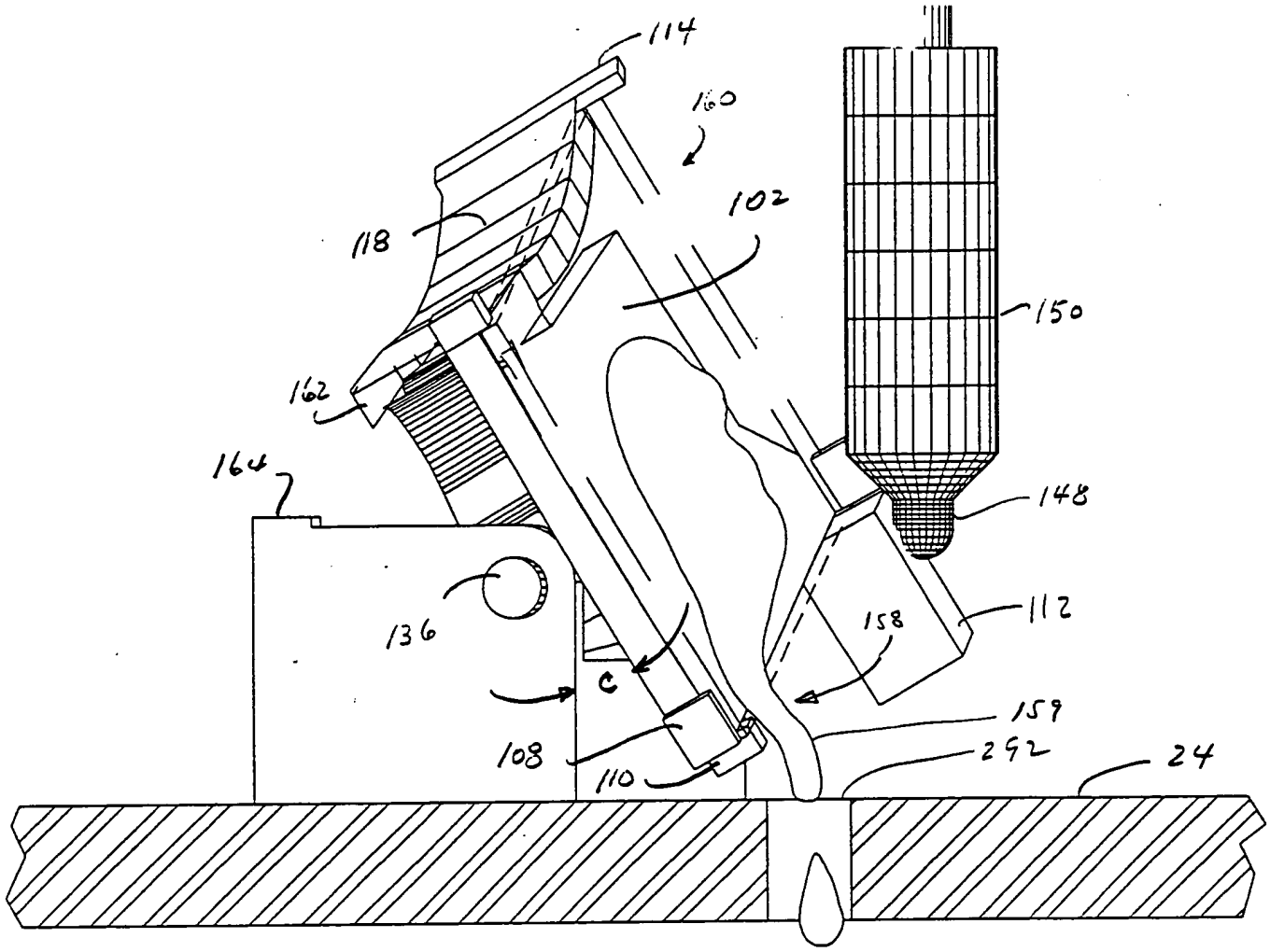
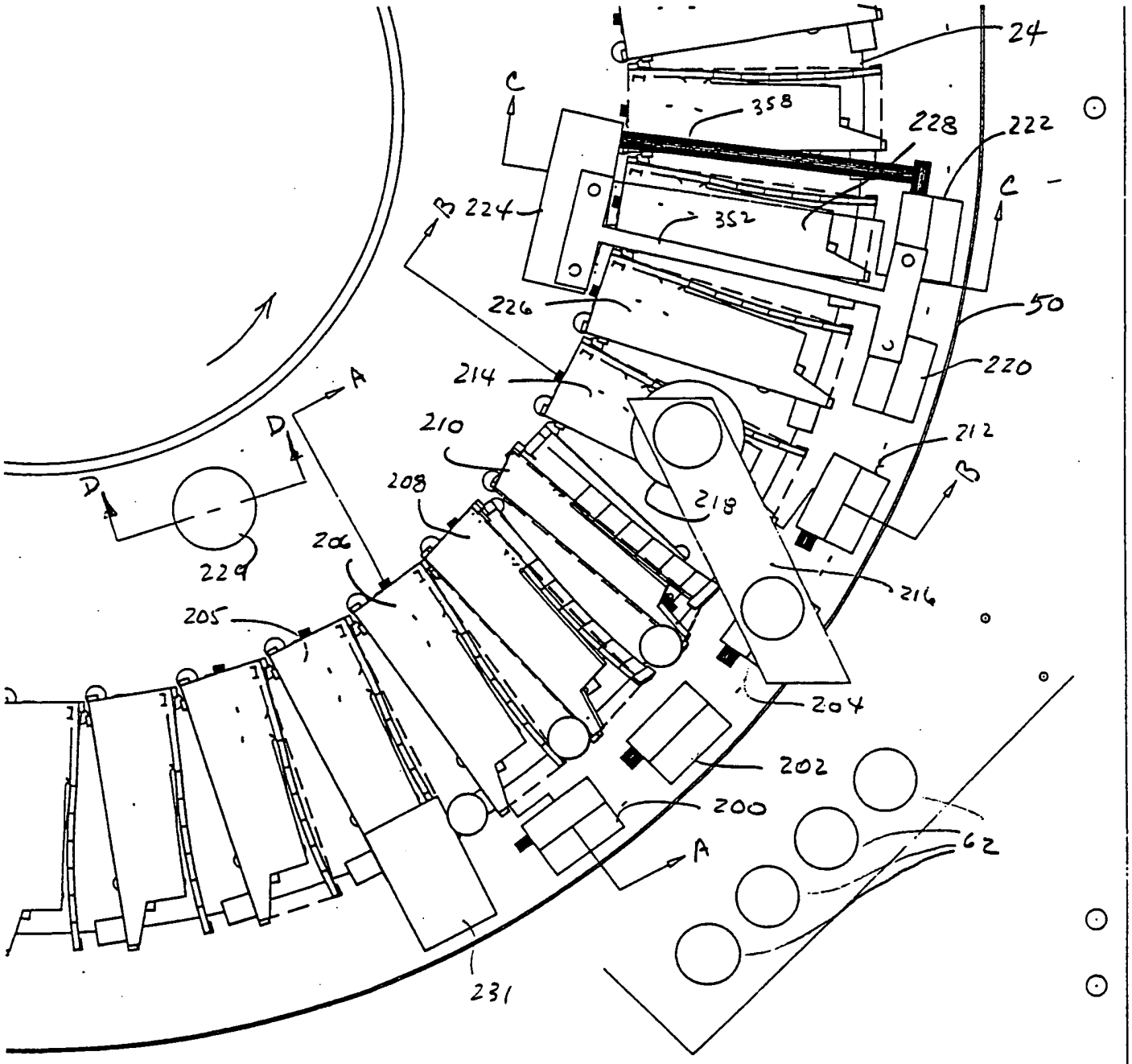


FIG. 10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D
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212
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204
202
200
62
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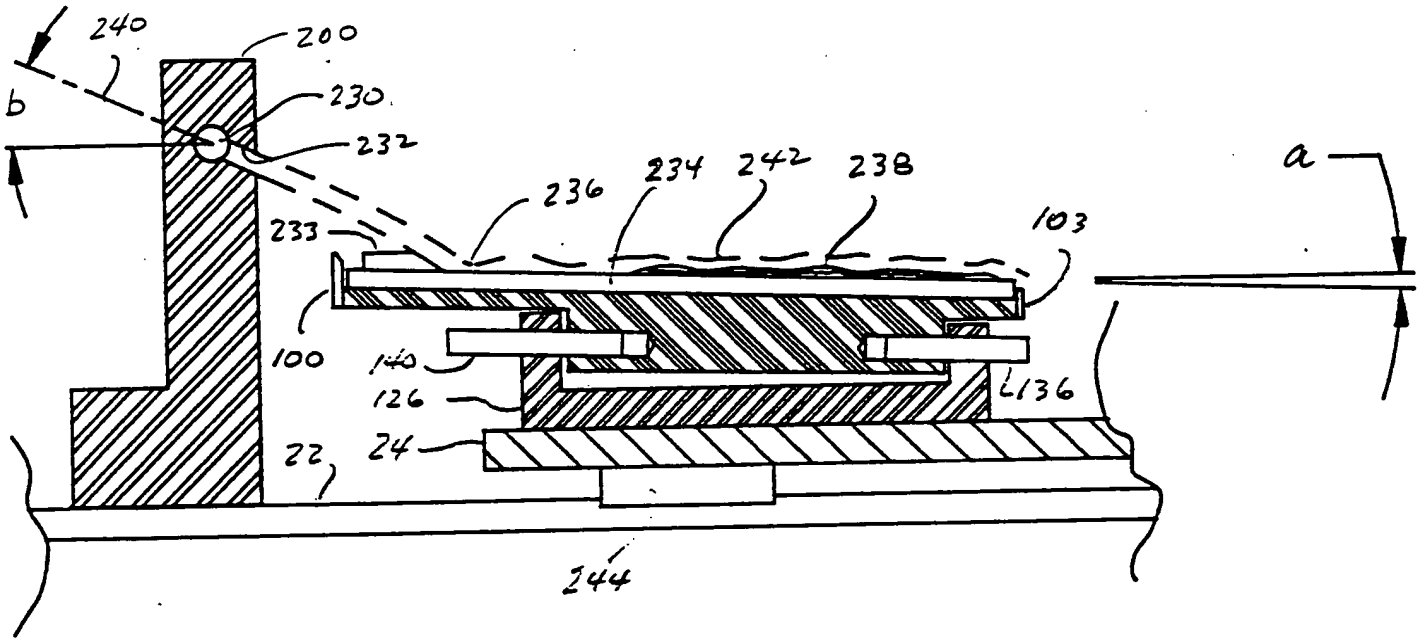
FIG. 11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233
236
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242
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103
a
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FIG. 12

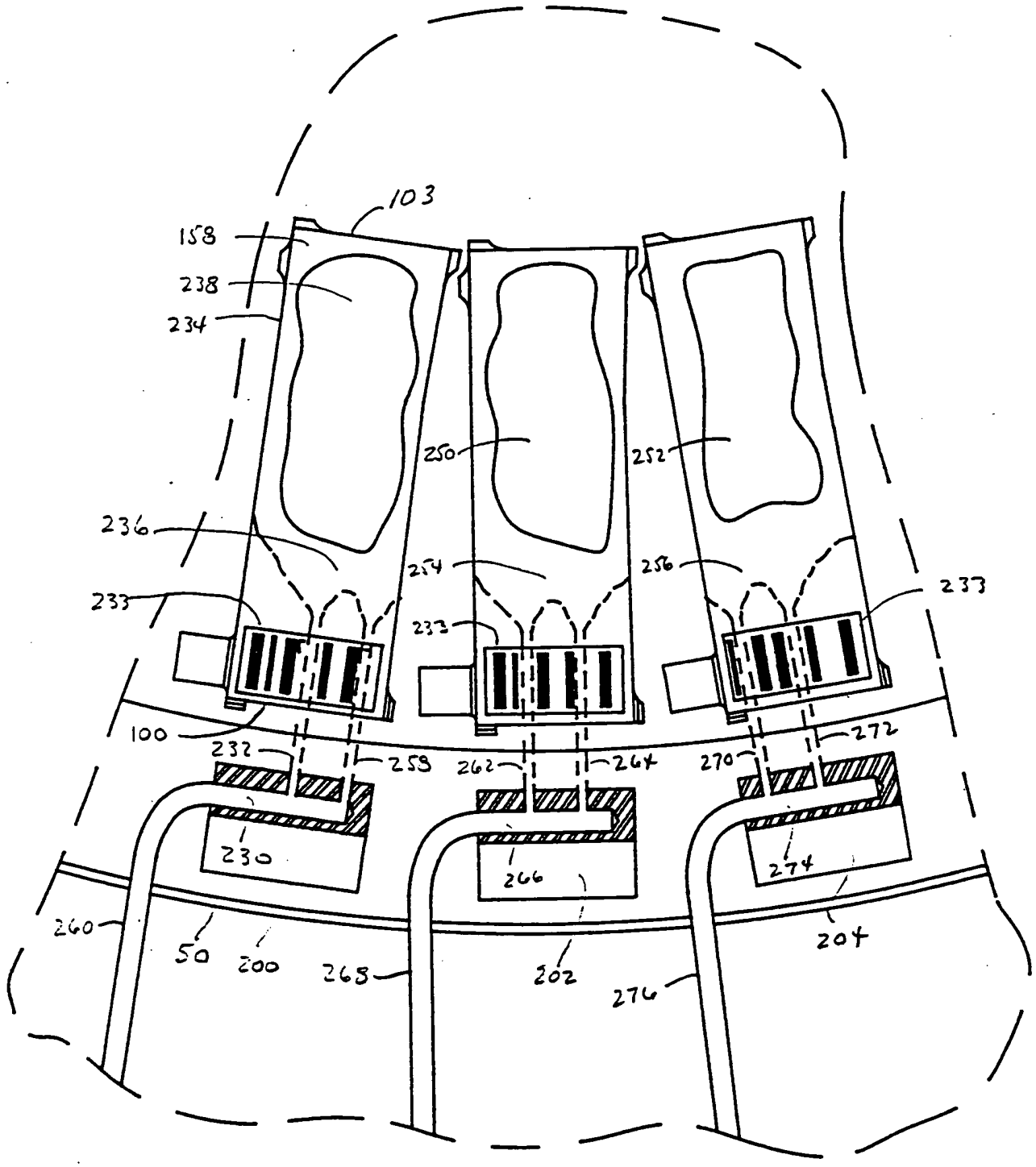
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FIG. 13

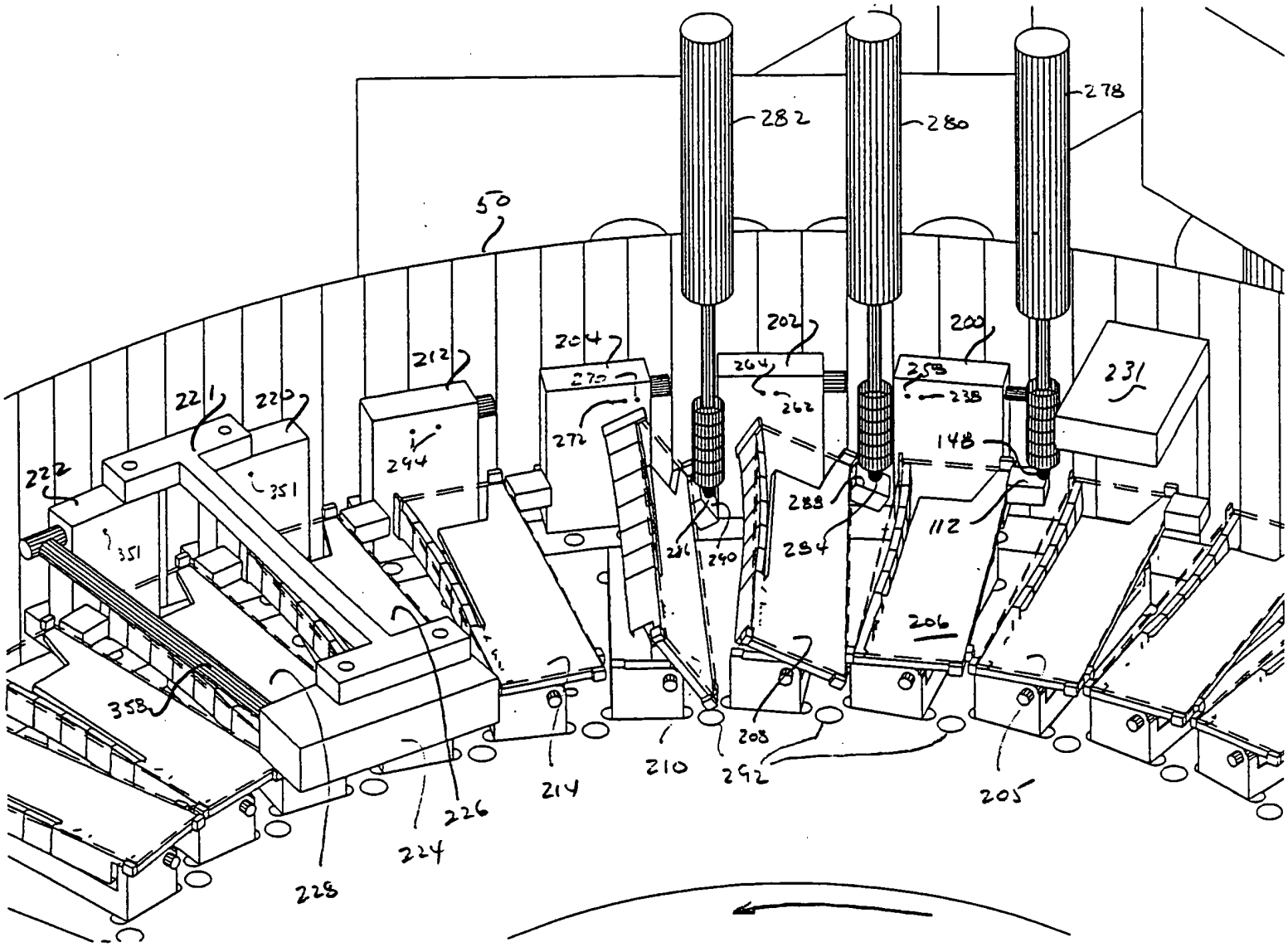
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FIG. 14

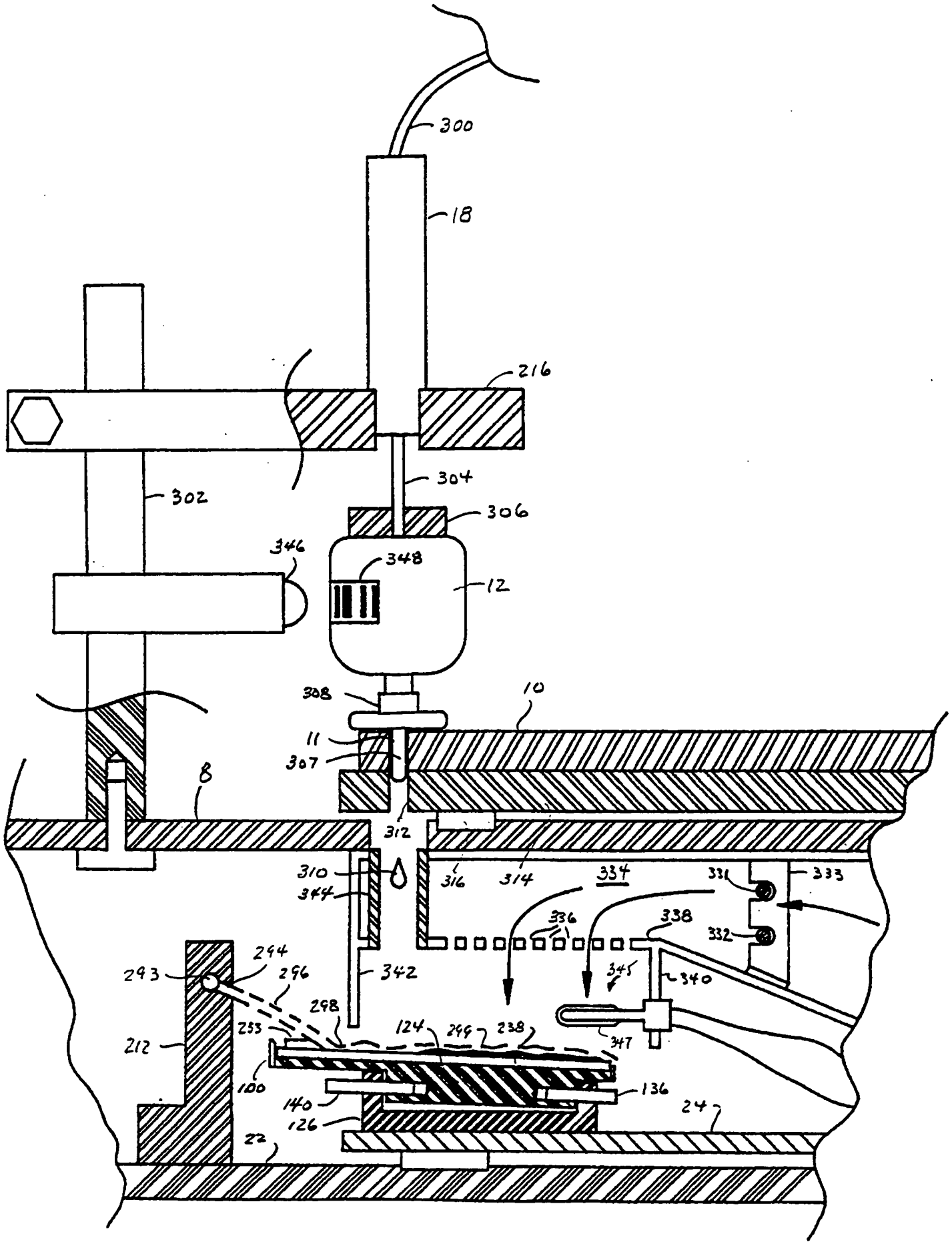
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FIG. 15

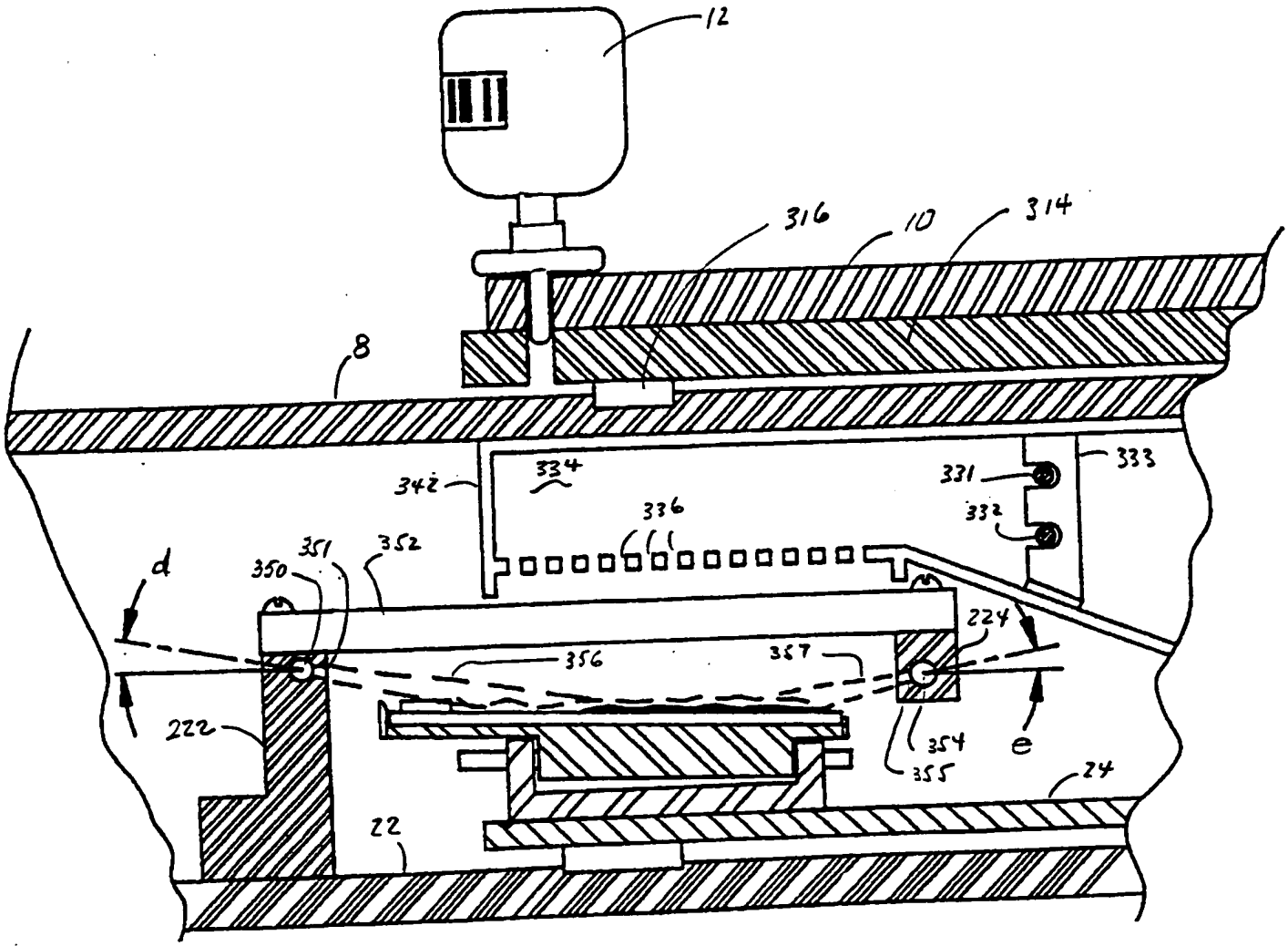
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FIG. 16

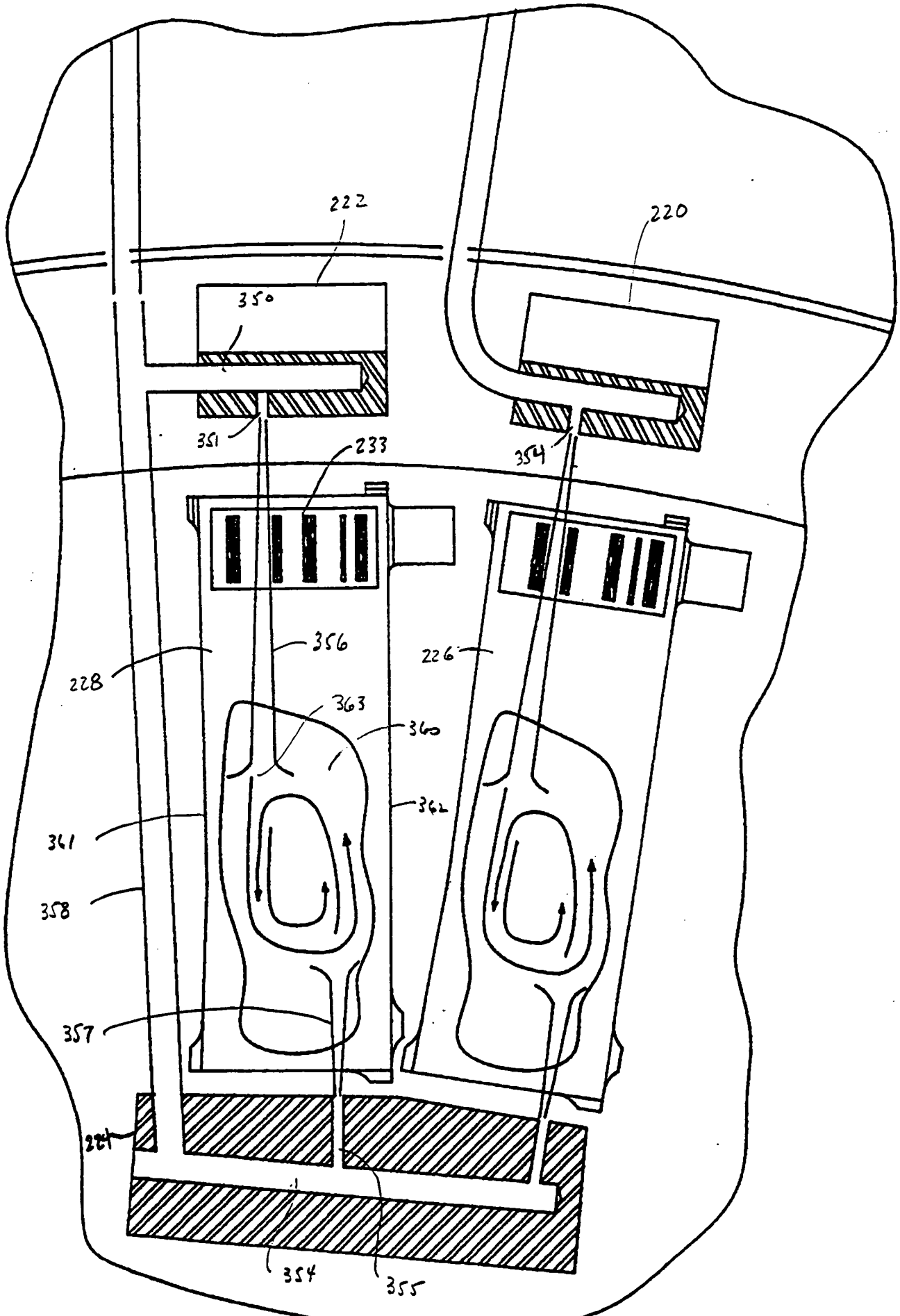
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FIG. 17

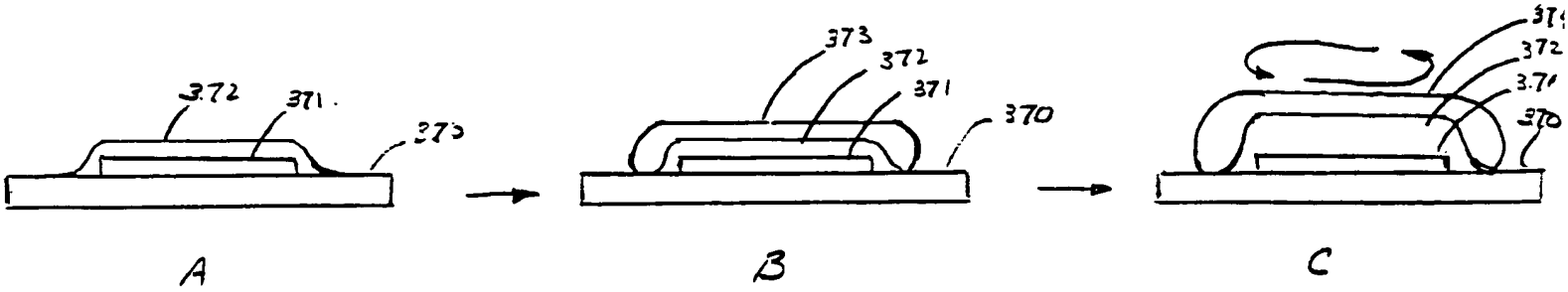

FIG. 18

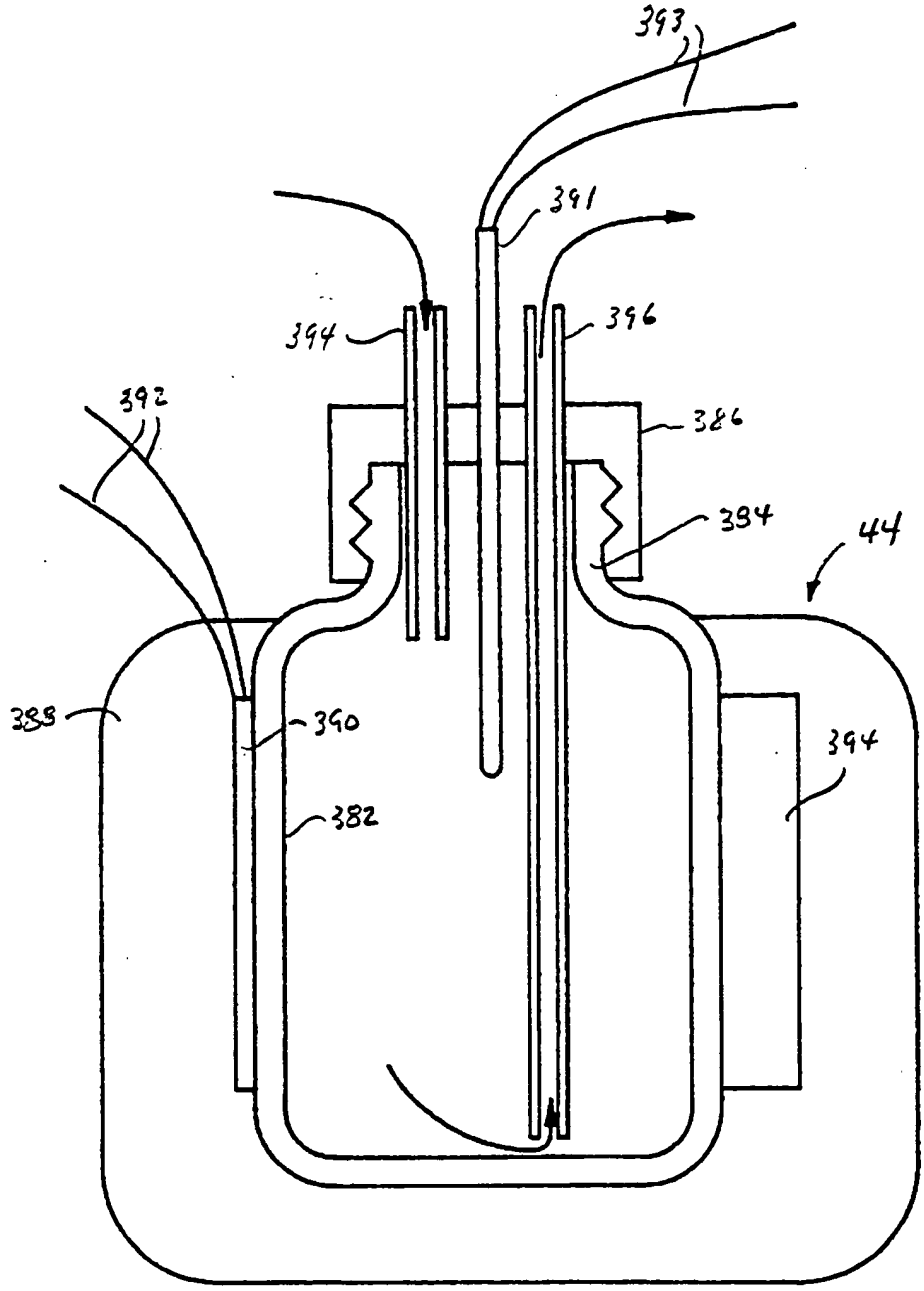

FIG. 19

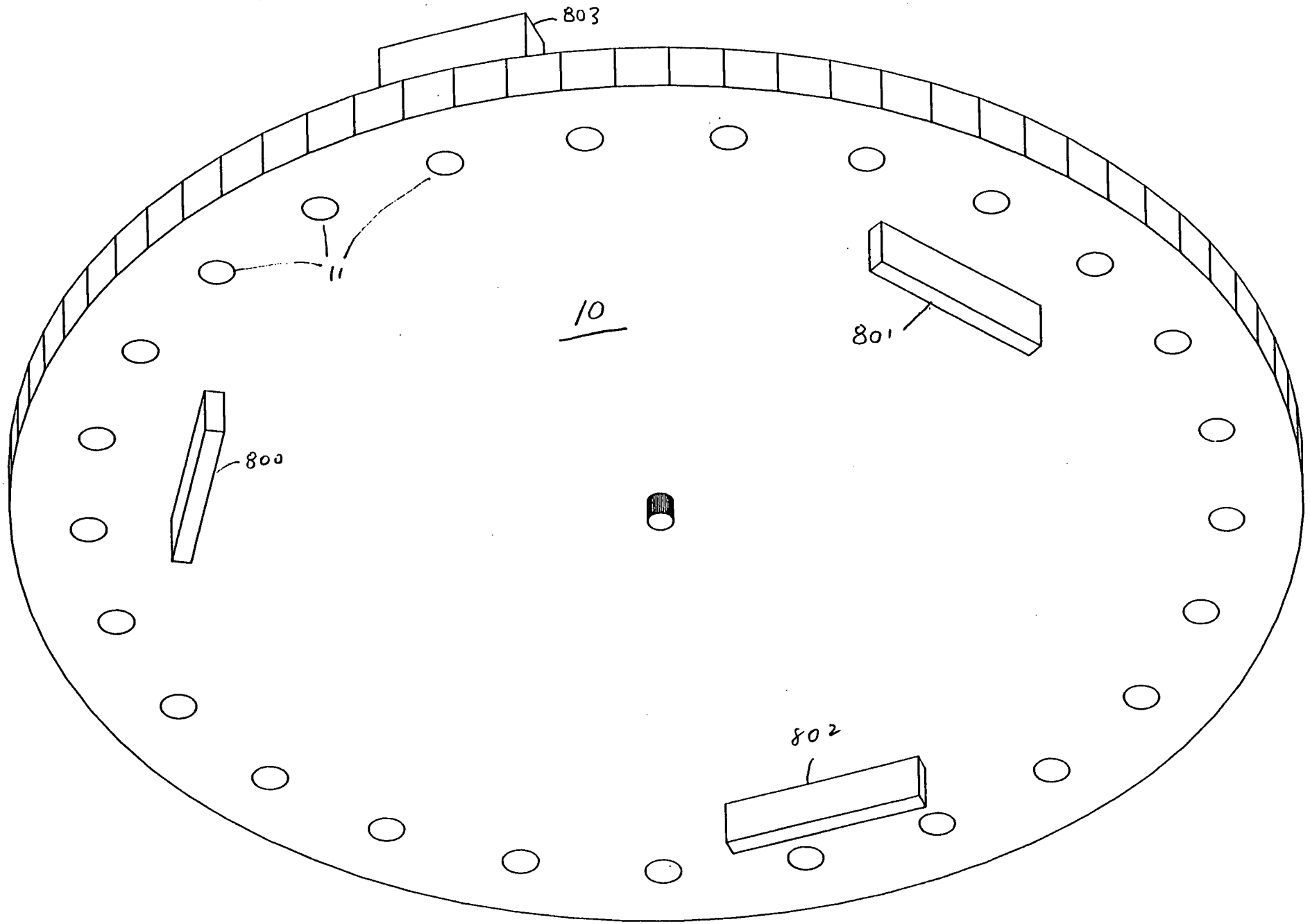

FIG. 20

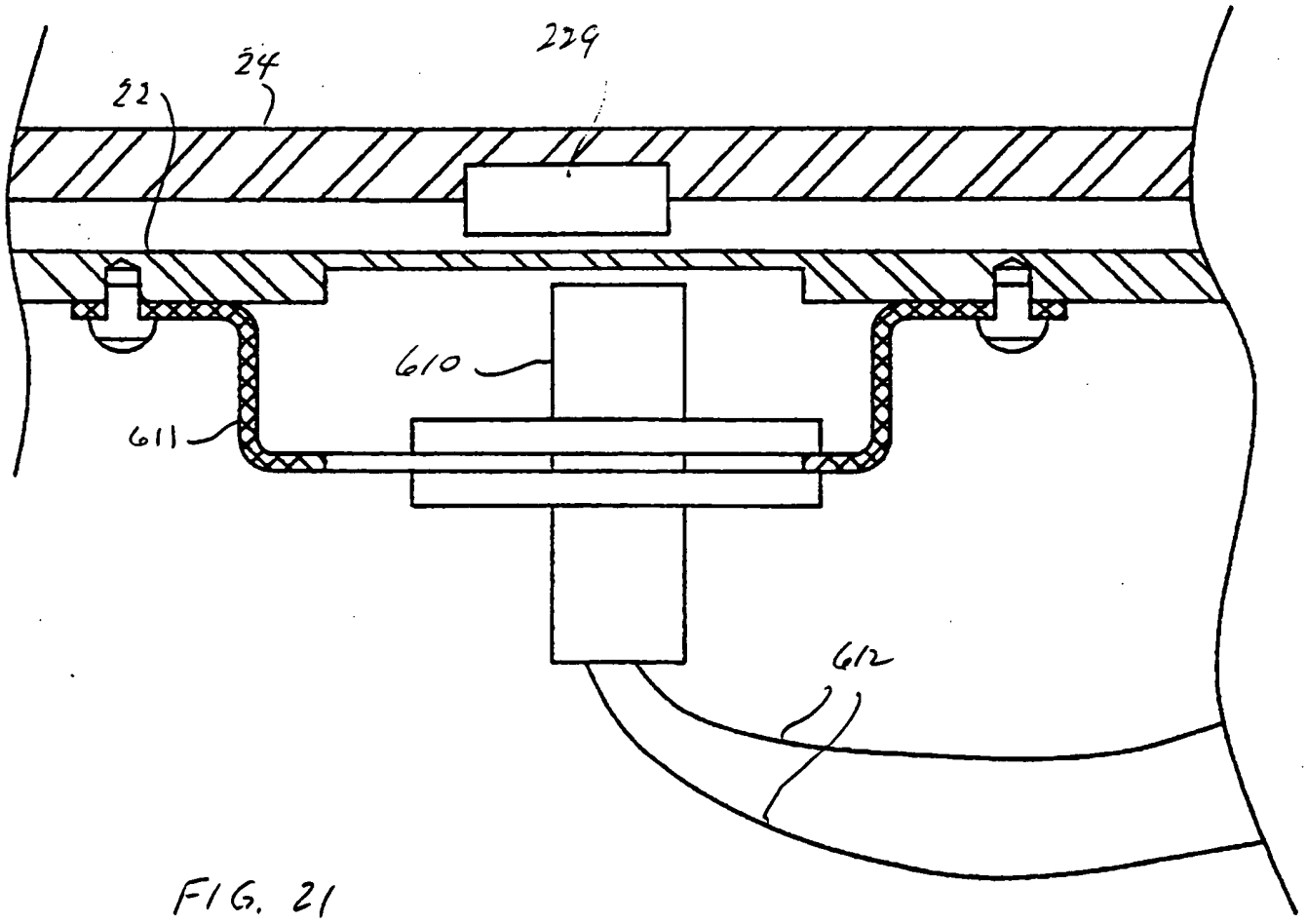

FIG. 21

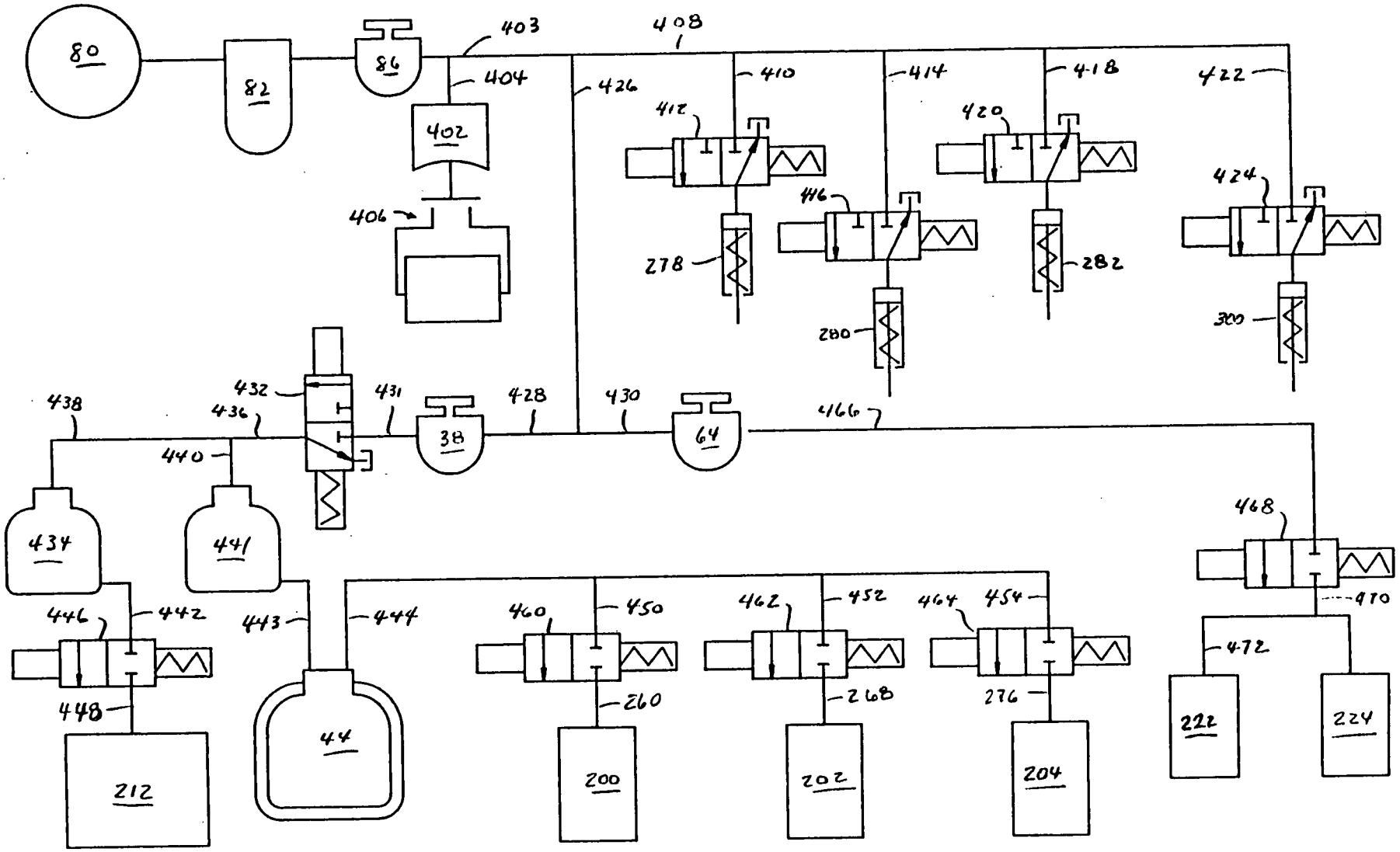
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FIG. 22

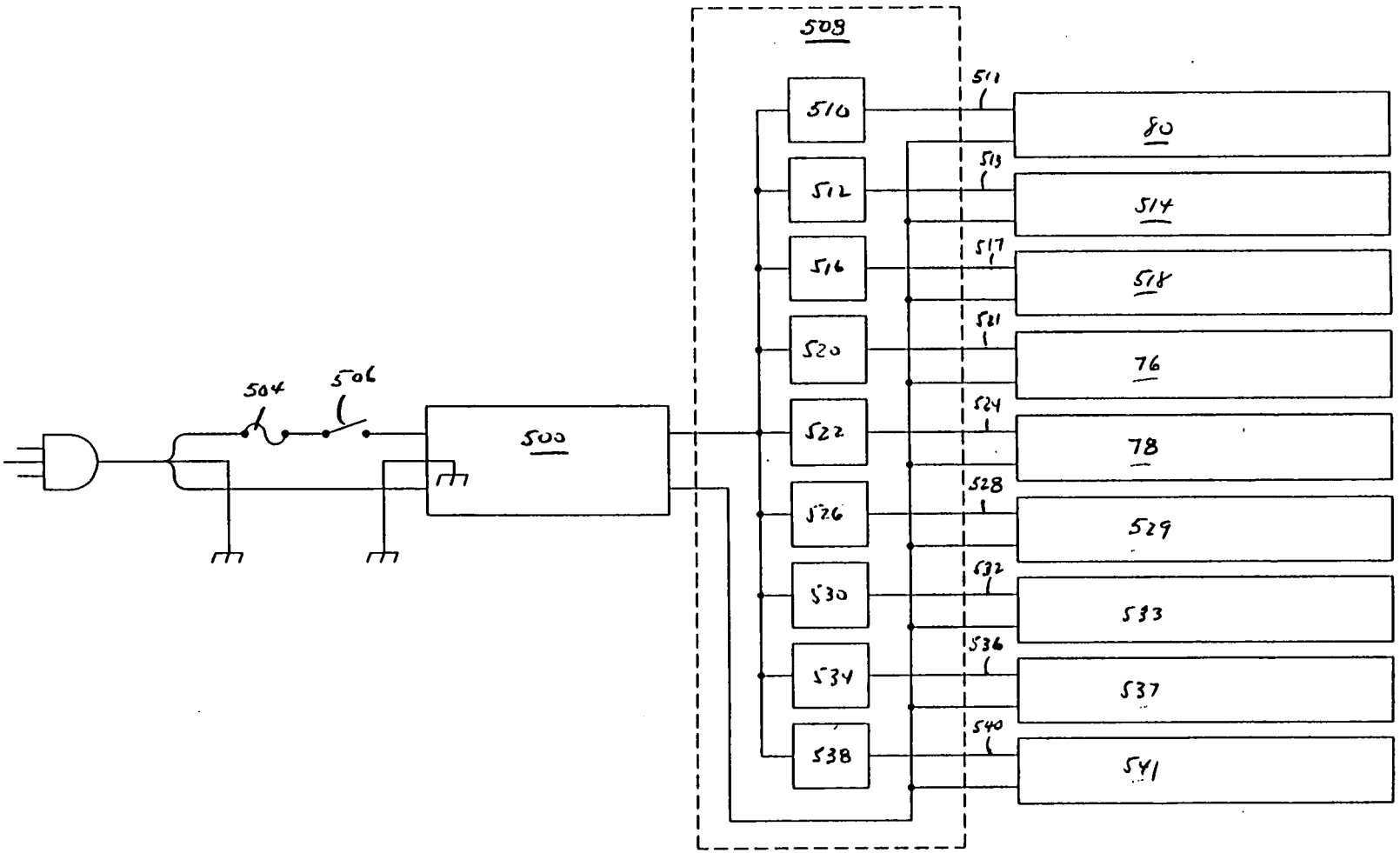
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FIG. 23

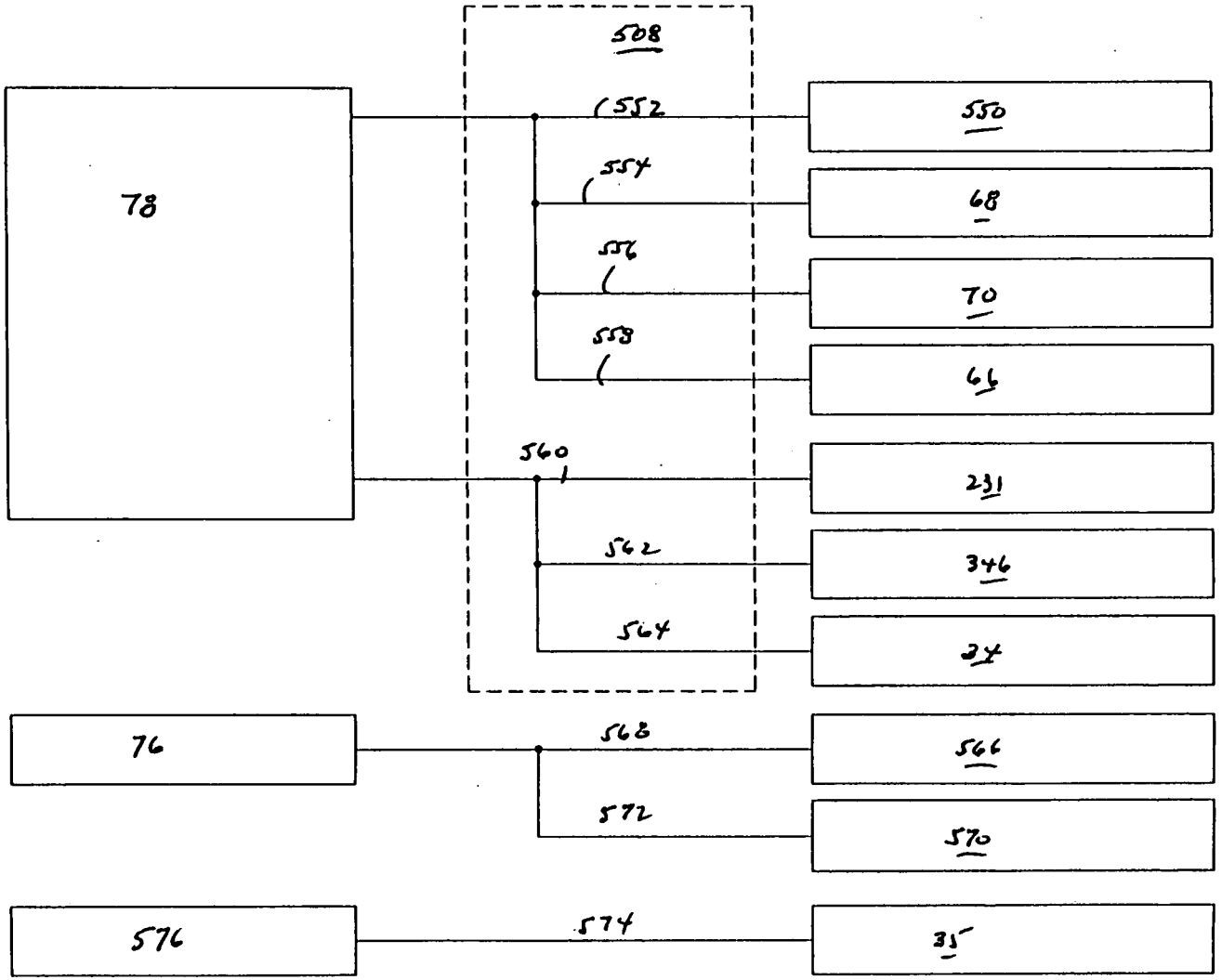
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FIG. 24

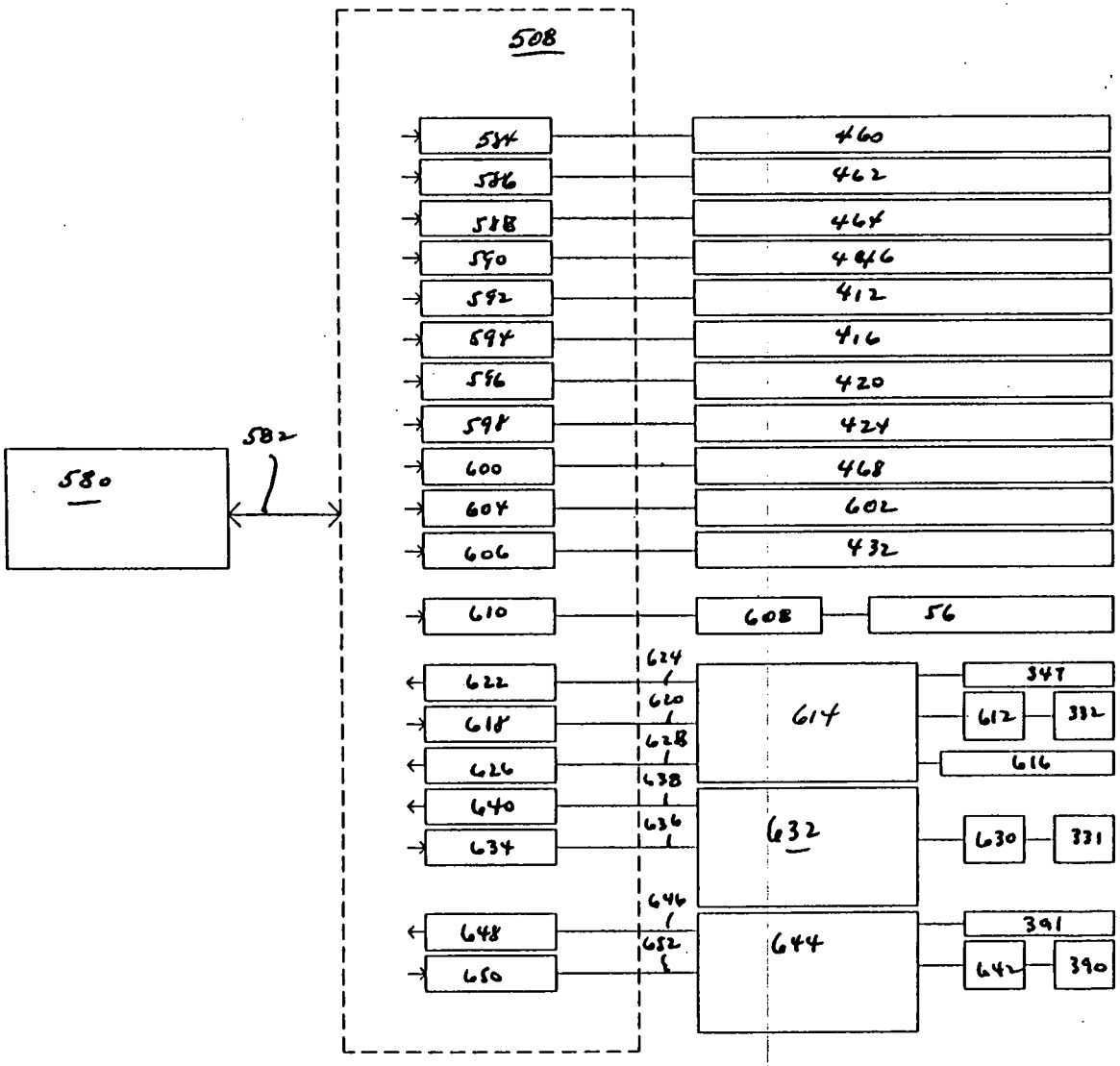
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FIG. 25

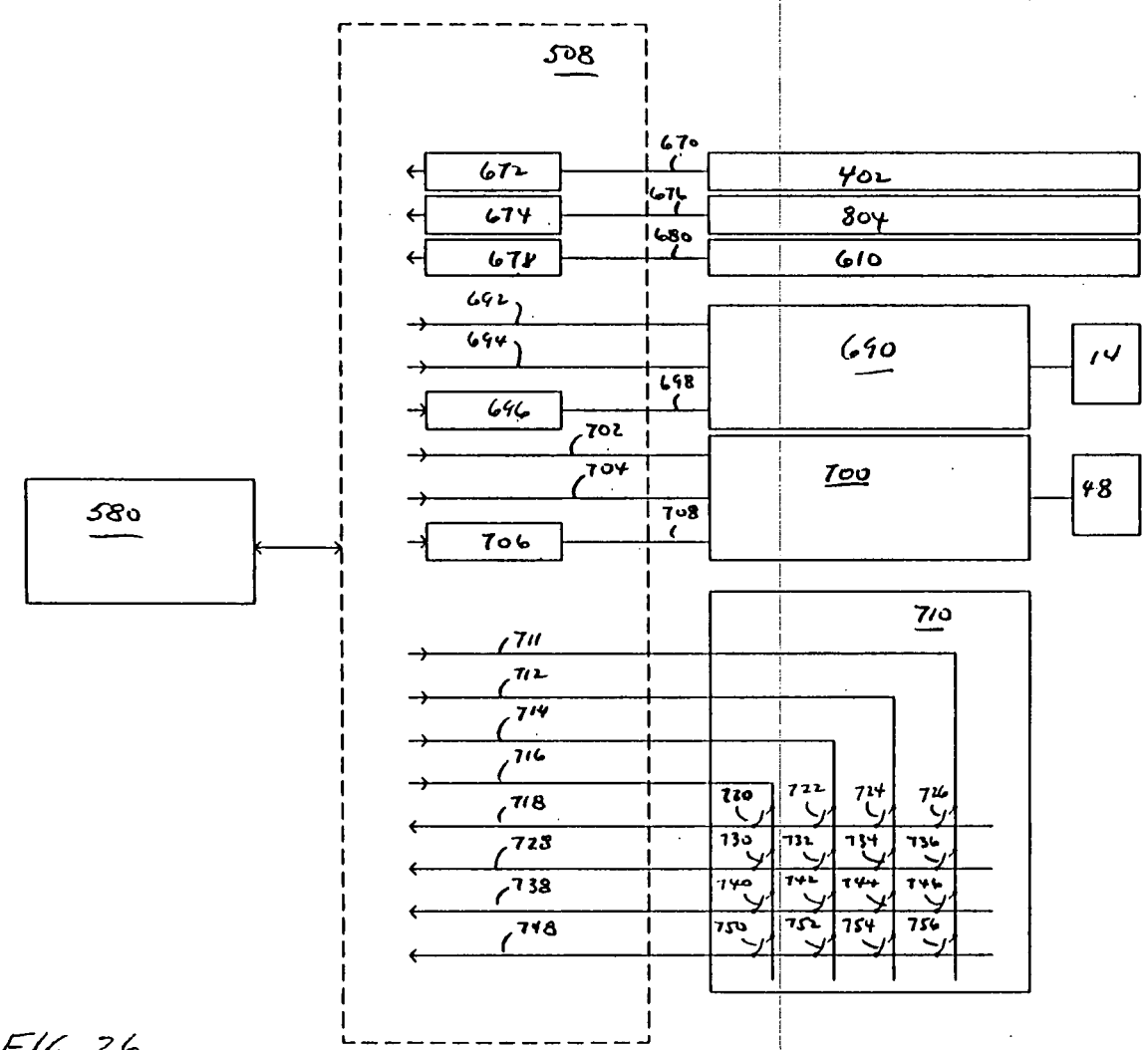
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FIG. 26

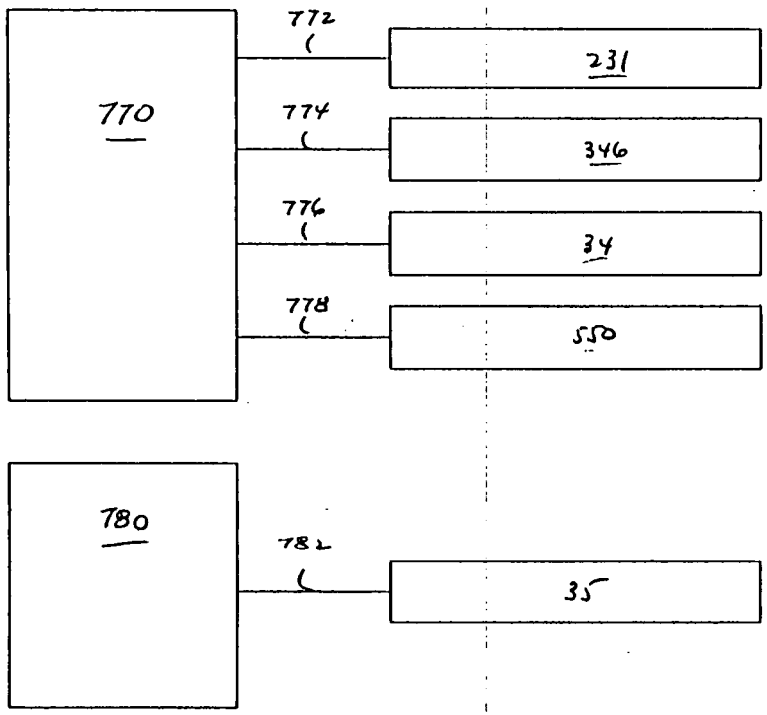

FIG. 27